EFFECT OF Si DOPING AND E-GLASS FIBER ADDITION ON PHYSICOCHEMICAL AND MECHANICAL PROPERTIES OF CALCIUM PHOSPHATE CEMENT

A THESIS SUBMITTED IN PARTIAL FULFILMENT
OF THE REQUIREMENT FOR THE DEGREE OF

Master of Technology

in

Ceramic Engineering

By

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Department of Ceramic Engineering

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CERTIFICATE

This is to certify that the thesis entitled, "**Effect of Si doping and e-glass fiber addition on physicochemical and mechanical properties of calcium phosphate cement**" submitted by Miss. Debasmita Pani in partial fulfillment of the requirement for the award of Master of Technology Degree in Ceramic Engineering at the National Institute of Technology, Rourkela (Deemed University) is an authentic work carried out by her under my supervision and guidance.

To the best of my knowledge, the matter embodied in the thesis has not been submitted in any other University/Institute for the award of any Degree or Diploma.

Date:

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ABSTRACT

Calcium phosphate cements (CPCs) are hydraulic cements. These are formed by a combination of one or more calcium orthophosphate powders. Calcium orthophosphates have been studied as bone re-pair materials for the last 80 years. Calcium phosphate cement (CPC) self-hardens to form hydroxyapatite, having excellent osteoconductivity and bone-replacement capability. The present study was aimed at preparation of alpha tricalcium phosphate and Si doped alpha tricalcium phosphate and studying the effect of addition of glass fibers into them. The physicochemical and mechanical properties of α-tricalcium phosphate (α-TCP) cement and Si doped α-TCP were studied. α-TCP was prepared by solid state sintering of $CaCO_3$, $CaHPO_4$ and Si doped α-TCP powders were synthesized by reacting mixtures of $CaCO_3$, $CaHPO_4$, and SiO_2(TEOS). Setting time, phase composition, hydrolysis conversion rate, microstructure, and diametral tensile strength (DTS) of undoped CPC and Si-doped CPC were studied and compared. Both initial and final setting time of the developed cement was delayed because of Si addition. Crystalline phases of HA (JCPDS 9-432), α-TCP (JCPDS 29-359) and β-TCP (JCPDS 9-169) were detected in the X-ray diffraction (XRD) pattern after setting and immersion in SBF for 0 hours to 10 days. The intensities of the α-TCP peaks at 22.2° (201) and 24.1° (161,-331) decreased when the time of immersion in SBF increased from 0 hours to10 days, due to its transformation into HA. Si doped CPC showed little slower rate of conversion into HA phase as compared to undoped CPC. The SEM image of the microstructure of cement showed better compactness and greater crystal entanglement in undoped CPC as compared to Si-doped CPC. This lower porosity and greater compactness in the microstructure attributes to greater DTS values observed in undoped CPC. Addition of 10 wt % of e-glass fiber into Si-doped α-TCP increased the mechanical strength of CPC as the fibers could make the structure compact and provide reinforcement.

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1. INTRODUCTION

1.1 What is Bone Cement

According to IUPAC bone cement is defined as " Synthetic, self-curing organic or inorganic material used to fill up a cavity or to create a mechanical fixation". Bone cement is used for fixation of prosthesis to the living bone in orthopaedic operations. It is also commonly known as PMMA (polymethylmethacrylate). It is constituted of mainly two components: (i)A Polymer (i.e. powder) and (ii) A Monomer (i.e. liquid). The above two components are in turn made up of from a mixture of different ingredients that determine the unique characteristics of the bone cement.

Bone cement to be considered effective should be durable over a period of time and should be able to withstand compressive stresses, bending stresses and transverse loads. As lesser porosity results in better mechanical strength, property of the bone cement should be such that lesser air gets entrapped into it due to mixing technique and/or due to its chemical composition.

Artificial joints can be successfully transplanted using bone cement. The bone cement is used to fill the free space between the prosthesis (i.e., the artificial joints) and the bone. Human hip is acted on by approximately 10-12 times the body weight. Hence the bone cement must provide elasticity so that it can absorb the forces acting on the hips to ensure that the artificial implant remains in place over a long period.

1.2 Calcium Phosphate Cement

CPCs were first discovered in the 1980s by Brown and Chow and Le Geros et al. The first commercial CPC products were introduced in the 1990s for treatment of fractures and maxillo-facial defects.

Calcium phosphates have been used by the biomedical and medical communities for several years[2].It eliminates the donor site pain as in the case of autografts or possible infection in the case of xenografts and allografts.

Calcium phosphate cements are used as synthetic bone grafts. It provides several advantages like biocompatibility, excellent bioactivity, osteoconductivity, injectability, resorbality and their ability to form a direct bond with bone.

Calcium phosphate cement (CPC) self-hardens to form hydroxyapatite which is the principle mineral component of bone. Calcium Phosphate cement can be easily implanted by using simple injection method or by other manual techniques for a variety of applications. Upon implantation CPC sets quickly under normal physiological conditions. It does not release any heat as it sets. However, CPCs also have some drawbacks such as poor mechanical performance, and lower strength due to its intrinsic porosity.

1.3 Classification of Calcium Phosphate Cement

- CPCs can be classified according to the end products developed namely (i) precipitated hydroxyapatite (HA) or (ii) brushite (DCPD). Hydroxyapatite is the most stable calcium phosphate at pH>4.2 and brushite at pH<4.2.

In Apatite cement setting reaction, a poorly precipitated crystalline HA and/or CDHA is formed as the end product. These reactions are carried out in an aqueous environment and have low crystallinity. These are much similar to biological apatite of bones and teeth. This similarity in properties are believed to be responsible for their excellent *in vivo* resorption characteristics.

Brushite cements form DCPD as their major end-product of the setting reaction. Brushite cements set by the acid-base reaction. During setting, the paste of brushite cement is always acidic. Acidic nature is required due to the following reasons (i) easier and faster preparation, (ii) better control of the chemical composition and reactivity, (iii) improved physio-chemical properties, such as longer setting times and larger tensile strengths due to a higher homogeneity. However, there are chances that the more acidic environment might reduce the biocompatibility of the cement formulation, due to low pH values during setting.

- CPCs can also be classified depending upon the number of components. CPC's can either be monocomponent or multicomponent.

In monocomponent CPCs, a single calcium phosphate compound such as alpha tricalcium

phosphate (α-TCP) hydrolyses to CDHA without varying the Ca/P ratio

$3\alpha\text{-}Ca_3(PO_4)_2 + H_2O \rightarrow Ca_9(HPO_4)(PO_4)_5(OH)$

In multicomponent CPCs two or more calcium phosphates, some of acidic nature and others of basic character, set following an acid–base reaction.

Apatitic cement: $2Ca_4(PO_4)_2O + 2CaHPO_4 \rightarrow Ca_{10}(PO_4)_6(OH)_2$

Brushitic cement: $3Ca_3(PO_4)_2 + Ca(H_2PO_4)_2.H_2O + 7H_2O \rightarrow 4CaHPO_4.2H_2O$

The basic component is generally tetracalcium phosphate (TTCP), and the acidic component can either be dicalcium phosphate anhydrous (DCPA) or dicalcium phosphate dihydrate (DCPD). The Ca/P ratio of the final HA depends on the ratio between TTCP and the acidic component.

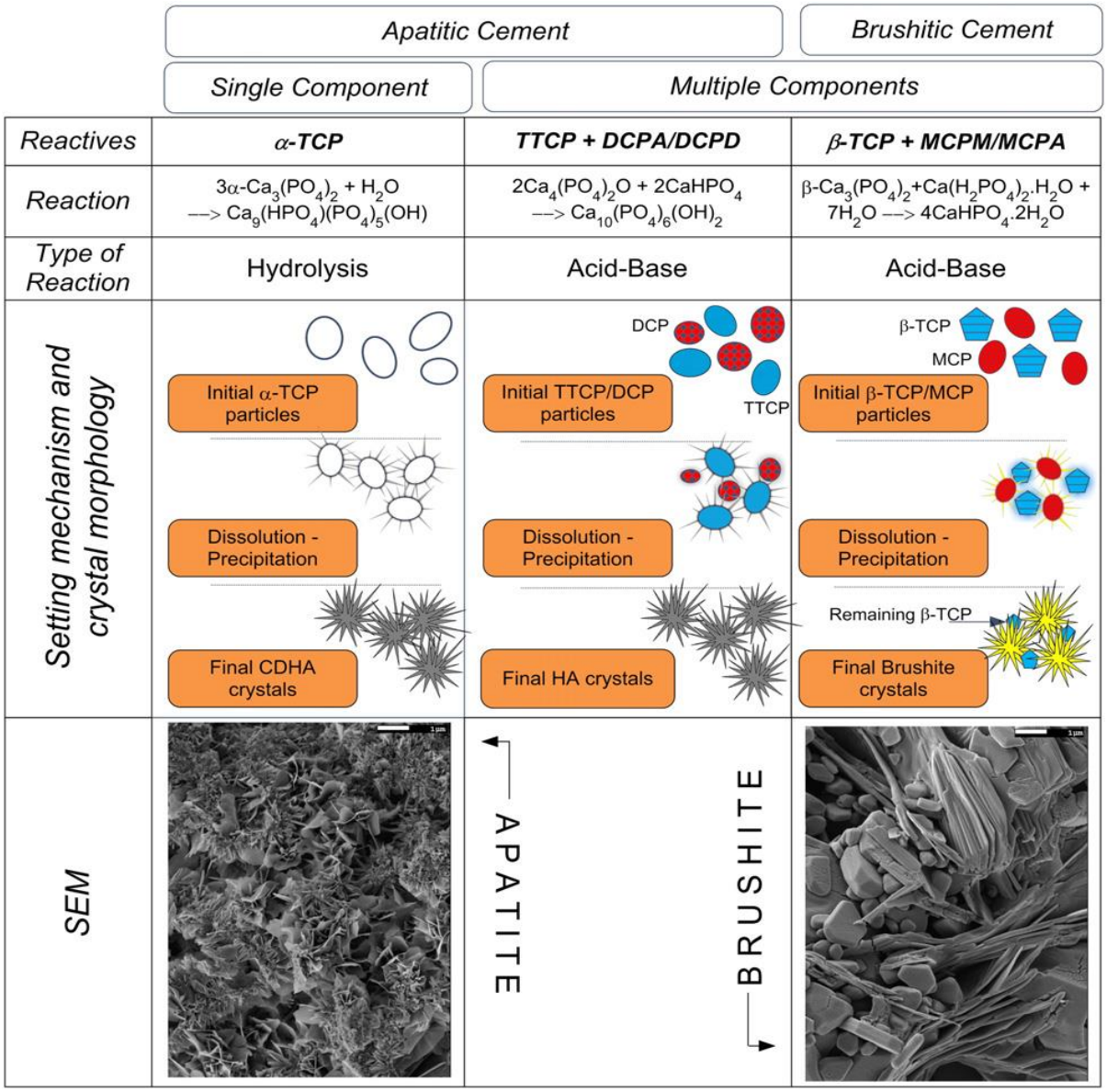

Figure 1: Classification of Calcium Phosphate Cement [3]

1.4 Injectable calcium phosphate ceramic

Hydroxyapatite is also known as Hydroxylapatite (HA). It is a naturally occurring mineral form of calcium apatite ($Ca_5(PO_4)_3(OH)$). It is usually written as $Ca_{10}(PO_4)_6(OH)_2$ to denote the two entities of its crystal unit cell. Hydroxyapatite is the hydroxyl end member of the complex apatite group. It has a hexagonal crystal structure. Pure hydroxylapatite powder is white in colour. Naturally occurring apatites can, however, be brown, yellow, or green.

Hydroxyapatite is the major natural building block of bone and teeth. Bone cements, comprising of calcium and phosphate precursors in an aqueous solution, initially forms a

paste and then hardens into hydroxyapatite bone cement. These are useful in fixing fractures and bone defects.

Hydroxyapatite is chemically similar to the mineral component of bones and hard tissues in mammals. Hydroxyapatite has a calcium to phosphorous ratio of approximately 1.67 which is generally the same as the calcium phosphate ratio in natural bone structures**.** It is amongst the few materials that are bioactive, i.e., they will support bone ingrowth and osteointegration. This justifies their use in orthopedic, dental and maxillofacial applications.

The chemical nature of hydroxyapatite makes it susceptible to substitution. Thus non-stoichiometric hydroxyapatites may also exist. The most common substitutions include carbonate, fluoride and chloride substitutions for hydroxyl groups. Defects can also exist due to formation of deficient hydroxyapatites. Dense hydroxyapatite ceramics possess the following features.

- The ability to integrate in bone structures and support bone ingrowth, without breaking down or dissolving (i.e. it is bioactive).
- Hydroxyapatite is a thermally unstable compound. Depending on its stoichiometry it decomposes at temperature of about 800-1200°C.
- Dense hydroxyapatite does not have ample mechanical strength to withstand load bearing applications for a long period.

Autogenous bone graft is generally used for reconstruction of bone defects and provides mechanical integrity to the skeleton system of the patient. However, bone reconstruction by autograft suffers from certain disadvantages for example, (i) need for a second surgery at the donor site, (ii) limited availability of shape of the bone required to be grafted, (iii) resorption of the bone graft [4-6].

Recently to reduce or eliminate bone grafting method injectable systems have been developed that can mould to the shape of bone cavity and can polymerize when injected in situ. Such systems render advantages such as lesser (i) time requirement to carry out the surgical operation, (ii) damage due to lesser muscle retraction, (iii) scars,(iv) post-operative pain. Poly(methyl methacrylate) (PMMA) is the most commonly used injectable bone cement. But factors such as its non degradability, high temperature requirement for curing can cause cell injury of the surrounding tissue [7].

In such conditions calcium phosphate cement (CPC) serves as an alternative injectable material. Injectability of CPC is required for the minimal invasive applications like, spinal

applications, vertebroplasty, bone void filling in closed fracture sites and the reinforcement of osteoporotic. CPC self-hardens to form hydroxyapatite.

Hardening: Self-setting calcium orthophosphate formulations are formed by mixing amorphous and/or crystalline calcium orthophosphate powder(s) with an aqueous solution such as distilled water, phosphate-buffered saline (PBS), aqueous solutions of sodium orthophosphate, orthophosphoric acid, citric acid, sodium silicate, magnesium hydro-orthophosphate or simulated body fluid (SBF). After the calcium orthophosphate powder(s) and the solution are mixed together, a viscous and moldable paste is formed that sets to a firm mass within a few minutes. When the paste becomes sufficiently stiff, it is introduced into a defect, where it hardens *in situ* within the operating theatre.

CPCs set as a result of a dissolution and precipitation process. The cement is considered to be set when it can resist a given mechanical load applied onto its surface. The entanglement of the precipitated crystals is responsible for cement hardening unlike acrylic bone cements, which harden due to polymerization reaction.

1.5 Properties::

CPCs provide

1. Self-setting ability *in vivo* .
2. Good injectability that allows cement implantation by minimally invasive surgical techniques, thus reducing damages caused by traditional surgical techniques.
3. Good osteoconductivity and occasional osteoinductivity.
4. Can be replaced by newly formed bone after a period of time referred to as (osteotransductivity).

1.6 Advantages and Disadvantages::

Some of the advantages of CPCs are listed below::

1) Moldability to perfectly fit the implant site, which assures good bone-material contact, even in geometrically complex defects.
2) Excellent biocompatibility and bioactivity.
3) No toxicity.
4) Low cost.

5) Ease of preparation and handling.
6) Setting at body temperature
7) Can be used to deliver antibiotics, anti-inflammatory drugs, growth factors, morphogenic proteins

CPCs also render following disadvantages:

1) Mechanical weakness: limited use due to potential collapse of material followed by soft tissue formation instead of bone formation (loaded areas). Until cements with adequate shear strength are available, most complex fractures that can be repaired with cement also will require metal supports.
2) Can be washed out from surgical defect if excess of blood flows.
3) Lack of macroporosity (especially inter-connected pores), which prevents fast bone ingrowth and the cements degrade layer-by-layer from the outside to the inside only.
4) The *in vivo* biodegradation of many formulations is slower than the growth rate of a newly forming bone

1.7 Applications::

- It is used in orthopaedic, dental and maxillofacial applications
- Calcium Phosphate Cement can be used to anchor artificial joints (hip joints, knee joints, shoulder and elbow joints)
- Repair of periodontal defects
- Augmentation of alveolar bone
- Sinus lifts
- Tooth replacement
- Repair of large bone defects caused by tumors [8-14]. They are also used as scaffolds in tissue engineering for bone or dentin regeneration [14-18].

2. LITERATURE REVIEW

CPCs were first discovered in the 1980s by Brown and Chow and LeGeros et al.. Studies on the use of calcium phosphates for bone defect repair appeared in the scientific literature as early as in 1920 The first commercial CPC products were introduced in the 1990s for treatment of fractures and maxillo-facial defects. CPCs were then used as efficient drug carriers between its inherent porosity.

In 1920, Albee and Morrison used "triple calcium phosphate" as stimulus for bone growth. The results indicated rapid bone growth and union in the bone deficient site. Then the next generation calcium phosphate cement materials such as ready-to-use injectable CPC, fiber reinforced (FRCPC), calcium phosphate-containing polymeric scaffold sets having greater efficacies and applications were produced[1]. Considering the on going researches on CPCs, replacement of many autografts currently in use by CPC type materials in future would not be an unrealistic goal.

Calcium orthophosphates have been used as bone repair materials for the past 80 years. In 1920 tricalcium phosphate (TCP) was implanted for the first time into animals to test its efficacy as a bone substitute[99].Hydroxyapatite (HA) was first implanted in rats and guinea pigs[100] in 1951. During 1970s, other calcium orthophosphates were synthesized, characterized, investigated and tried in medicine[101-107].

Calcium phosphate cements are also referred to as calcium phosphate bone cements (CPBC)[108]considering their suitability for repair, augmentation and regeneration of bones. Self-setting calcium orthophosphate formulations are mixtures of amorphous and/or crystalline calcium orthophosphate powder(s) with an aqueous solution such as distilled water, phosphate-buffered saline (PBS), aqueous solutions of sodium orthophosphate (~ 0.25 M), orthophosphoric acid, citric acid (~ 0.5 M)[110], sodium silicate[111,112], magnesium hydro-orthophosphate[113] or revised simulated body fluid (rSBF)[114]. They show good bioresorbability and are considered as the second generation bone substituting biomaterials[109].

Biocements, in case of defects, aim at imitating the bone until a new bone has been grown without causing much disturbance to the bone functions and properties. Initial setting of the cements also provides much needed mechanical strength until recovery of the defect. Self-setting calcium orthophosphate provides faster setting time; enhanced moldability and biocompatibility; and easy manipulation by incorporating additives[115] such as Na^+, K^+, Mg^{2+},

Ca^{2+}, Sr^{2+}, H+ , $PO4^{3-}$, HPO_4^{2-}, $H_2PO_4^-$, $P_2O_7^{4-}$, CO_3^{2-}, SO_4^{2-}, HSO_4^-, Cl^-, OH^-, F^-, SiO_4^4. Setting of calcium orthophosphate cements starts with its dissolution in an aqueous medium. During precipitation new crystals are formed that grows and forms a web of intermingling microneedles or microplatelets providing mechanical strength to the cement after setting. The setting reaction may also occur owing to hydrolysis of metastable calcium orthophosphate in aqueous medium. In this case Ca/P ionic ratio could be maintained. The chemical reactions during setting depend on the chemical composition of the cement.

The hydration process being slightly exothermic is beneficial for biomedical applications. This process undergoes five periods: initiating period, induction period, accelerating period, decelerating period and terminating period[117].Calcium Orthophosphate Cements are classified as either apatite (forming HA and/or CDHA as the end product)or brushite (forming DCPD as the end-product) of the setting reaction. Injectability is influenced by particle size (smaller) and shape (spherical shape or round). Lack of cohesion in cements may prevent its setting. Porosity is required for good in vivo resorption of implanted materials. Calcium orthophosphate cements have good compressive strength[118] but this value is lower than that of natural bones, teeth or sintered calcium orthophosphate bioceramics[116].

Self-setting calcium orthophosphate formulations have been successfully used for treatment of calcaneal fractures[120], hip fractures[121,122], augmentation of osteoporotic vertebral bodies[123], distal radius fractures[124], tibial plateau fractures[138,124-128], restoration of pedicle screw fixation[129,130], reinforcement of thoracolumbar burst fractures[131], cancellous bone screws[132,133], in wrist arthrodesis[134] and for fixation of titanium implants[135]. In the cement formulations, drugs might be incorporated into both a liquid and powder phases. Crystal morphology[136] influences the drug delivery properties of the cements.

Unlike CPCs, most of the other synthetic materials prepared are unable to adapt to the exact shape of the bone defect due to which the contact between the implant and surrounding tissues is not proper. It is expected that the use of self-setting calcium orthophosphate formulations will enable a faster recovery. These biomaterials give equal contribution to the biological and materials sectors. Therefore, more versatile ideas and approaches are required for its further development.

Amorphous calcium orthophosphate is also referred to as ACP. Interest in ACPs arose in the 1970s, due to their possible presence in the bone of vertebrates though it is not detectable in the earliest stage of tissue formation [86,87,88,89–94]. The CaP phases in milk have some analogy with ‘pure’ ACP phases. Amorphous calcium polyphosphates contain concatenated phosphate

groups. ACP phases are one of the most frequent forms of CaP minerals found in biological organisms[85]. CaPs when synthesized through precipitation process generally produce ACP as the intermediate phase. ACP is responsible for the setting reaction in self-setting injectable cements.

ACPs are considered as the reservoir of calcium and phosphate ions in many biological systems, for example in primitive organisms. They can be found in teeth and the exoskeletal structures of marine invertebrates[90] abundantly. The existence of ACPs in vertebrates is mainly present in the inner ear structures of embryonic sharks and milk of mammals[86,90]. Recent works have shown presence of a transient amorphous mineral precursor in bone and teeth. Characterization of these transient amorphous mineral phases in a tissue without altering them by dehydration, irradiation or the use of solvents is difficult. It transforms into amorphous-like domains on dehydration. Whereas wet and/or poorly preserved amorphous phase samples containing hydrated surface forms more stable apatite or octacalcium phosphate (OCP) phases.

ACPs are distinguished by their Ca/P atomic ratio. Amorphous tricalcium phosphate (ATCP) is formed at high temperature and has atomic Ca/P ratio of 1.5 in a pH range of 9 to 11. ACPs can contain HPO_4^{2-} ions instead of PO_4^{3-}in case of acidic solutions and possess a lower Ca/P ratio. ACP readily converts into dicalcium phosphate dihydrate (DCPD) with lower Ca/P ratios of 1.15 due to its unstability.

ACPs can be prepared either in aqueous medium at low temperature (known as wet route) or by using high energy processing or at high temperatures (known as dry route). Ca/P ratio of synthesised ACP depends these routes. The ratio may range from1 to 2 or may be even higher. The ACP synthesized should have a Ca/P ratio of 1.5 or 1.33.

ACPs are used to form coatings, cements, ceramics and composites. Presence of ACP is sometimes controllable and sometimes uncontrollable as in plasma sprayed HA coatings. ACP being a resorbable cements is used for drug delivery of antibiotics or growth factors for orthopaedic and dental applications [95].

The instability of ACPs restricts it from its mass production, storage and processing. Though its adsorption properties are not well known its ability of easy assimilation in vivo can be used to prepare composites with high remineralising potential or drug carriers.

Tricalcium phosphate is available in three polymorphic forms: low-temperature form i.e., β-TCP and the high-temperature forms i.e., α-TCP and α'-TCP. α' is not much significant as it exists at temperatures >~1430 °C and reverts back to α-TCP on cooling below the transition

temperature almost instantaneously. β-TCP is more stable at room temperature and transforms reconstructively[49,50] at temperature ~1125°C to α-TCP, and can be retained during cooling to room temperature[51].

α- and β-TCP are currently being used in dentistry, maxillo-facial surgery and orthopaedics. Several commercial mono- or biphasic bioceramics and composites have β-TCP as their major component whereas α-TCP is the major constituent of various hydraulic bone cements[52,53].

α- TCP and β-TCP have different biological properties and clinical applications. This is because though α- TCP and β-TCP have same chemical composition, they differ in their structure, density and solubility. Both α- TCP and β-TCP are used in clinical operations. α-TCP crystalline structure was related to the mineral glaserite ($K_3Na(SO_4)_2$) by Dickens and Brown in 1972[54] and later studied in detail by Mathew et al. in 1977[55] and more recently by Yashima and Sakai[56]. α-TCP crystallizes in monoclinic crystal system.

Synthesis of α-TCP can be carried out by thermal transformation of a precursor with molar ratio of Ca:P = 1:1.5. Self-propagating high-temperature synthesis[71] and combustion synthesis[57–59] have also been employed for α-TCP synthesis. The biological behaviour of α-TCP-based biomaterials has been studied in several in vitro[60–64] and in vivo[65–70] studies. α-TCP is non toxic and non irritant on intact rabbit skin[70] in solid and paste forms. New bone deposition was observed on the surface of α-TCP by the experimental groups and fibrous connective tissue was predominantly present at the centre of control sites. α-TCP is thus a degradable osteoconductive material, used for bone regeneration[66]. α-TCP particles are also efficient drug carriers.

Thermal transformation of β-TCP above the temperature resulting in polymorphic transformation of β→α is the rational way to synthesize α-TCP. α-TCP is thermodynamically metastable at temperatures < ~1100°C but as the polymorphic transformation of β→α is reconstructive with considerable activation energy α-TCP may be preserved at this temperature without quenching. α-TCP has been proposed as component of several bone cements since last two decades. Several granules, blocks and composites have been synthesized from α-TCP since then which have received growing attention as bone repairing materials. However, commercial α-TCP, reagent or biomedical grade, are very scarce so researchers and developers have to synthesize it themselves.

CPCs are biocompatible, bioactive, osteoconductive and osteointegration[137–139]. Mostly CPC systems based on α-tricalcium phosphate (α-TCP) or tetracalcium phosphate (TTCP)[140–150] are studied. These systems have slow setting reactions creating problems in surgical

operations. Upon setting these calcium phosphates systems are converted to apatite. In apatite phase, the CPC crystals grow progressively and gets entangled. This renders rigidity and strength to the set cement.

α-TCP hydrolyzes in water, to form calcium-deficient hydroxyapatite (CDHAp). TTCP gets completely converted to apatite by acid–base reaction with a neutral or acidic phosphate As apatitic cements are sparingly soluble under physiological conditions, their process like bioresorption and complete substitution by new bone are very slow. Faster setting time for the above CPC systems can be achieved by the addition of highly reactive calcium phosphates with reduced particle size and crystallinity, for example addition of amorphous calcium phosphate (ACP). This could also enhance the bioresorption of the resultant formulations. Precipitated ACP has a high specific surface area but lacks long-range periodic atomic-scale ordering of crystalline HA. Larger specific surface area causes faster resorption and also enhances osteoblast precursor cell attachment.

During hardening of CPC-ACP cement, it is converted into nanocrystalline apatite and gets fully resorbed and replaced by bone after 26 weeks of implantation. Though ACP/DCPD cement paste does not harden at room temperature appreciably, but it hardens very fast at body temperature thus ensuring faster setting upon implantation.

Addition of ACP to α-TCP and TTCP/MCPM causes surface transformations which enables better cell attachment than ACP-based CPC; and also modifies their microstructure forming smaller crystal which reduces its compressive strength. The set ACP/α-TCP-based cement forms nanosized needle-like particles, while the set ACP/TTCP/MCPM-based cement forms a dense conglomerate of nano spheres. This nanocrystalline apatite formed promotes cell viability and proliferation on the cement surfaces. ACP addition decreased the crystallinity of the end products. Further studies are required to understand the effect of surface crystal structure and transformation of cement surface in cell adhesion and metabolism.

CPCs are hydraulic cements generally formed by combination of one or more calcium orthophosphate powders mixed with a liquid phase. Unlike conventional calcium phosphate ceramics, CPC has an advantage that it can harden in vivo, through a low-temperature (at room or body temperature)setting reaction. But due to the intrinsic porosity of CPCs, calcium phosphate ceramics have greater mechanical strength than it limiting its application in non- or moderate-load-bearing situations[19,21]. CPCs also lack full injectability and has slow resorption rate. There is not heat release during the setting reaction of CPCs. Hence, different drugs and biological molecules can be incorporated into them making them suitable for drug

delivery applications [20]. CPCs have excellent bioactivity, osteoconductivity and resorbability (resorption rate depends on their composition and microstructure) which makes them a better choice as drug carriers than to acrylic bone cements and other injectable biomaterials and polymers. Though intrinsic porosity of CPCs lowers its mechanical strength but it allows the incorporation of drugs, biologically active molecules and even cells, without any thermal denaturalization or loss of activity during preparation or implantation.

Efficiency of material as a drug delivery device depends upon its microstructure i.e. their specific surface area, permeability, tortuosity and porosity; the degradation potential of the matrix, the solubility of the drug and the nature of the interactions between drug and matrix. Usually drugs are incorporated into CPCs by (i) blending drug powder with the solid phase (ii) dissolving it within the liquid phase(iii) incorporation of the drug by impregnation of pre-set CPC solid blocks or granules with a drug solution (iv) incorporation of the drug on polymeric microspheres before blending with CPC. In the first two cases the drug is incorporated throughout the whole volume of the material, although incorporation in the liquid phase provides a more homogenous distribution. In the third case, hydrated compounds with high specific surface areas and particular microtextures that supports drug loading and release mechanisms can be obtained due to material consolidation by a low-temperature dissolution-precipitation reaction though injectability is compromised in this case. In the last case of drug incorporation modification of the release kinetics of the drug (limiting burst release) can be done. Moreover in this case the degradation of the microspheres creates a porous matrix that can enhance resorption and remodeling capability.

The addition of a drug can modify the clinically relevant properties of CPCs like its setting kinetics, rheological properties, mechanical properties and microstructural development. Distribution of the drug within the CPC determines its drug release kinetics. When the drug is incorporated in the powder phase of the CPC, partial dissolution of the drug particles takes place. This depends on the solubility of the drug in the reactant liquid phase of the CPC.

Several works have dealt with the incorporation of different active principles namely low molecular weight drugs, high molecular weight biomolecules and ions; that aim at achieving local and controlled delivery to bone or dental sites.

To allow a more systematic study it is desirable to improve the homogeneity of the methods evaluating release and in-depth study of the release mechanisms.

Many discoveries and developments have been made in the field of CPCs starting from calcium silicate and sulphate based cements in the construction industry to poly(methyl

methacrylate) medical cements. Basic properties of CPCs such as setting time, injectability and cohesion require further research.

It is known that certain factors such as (i) powder size (smaller size-shorter setting time), (ii) amount of liquid (smaller amount-shorter setting time), (iii) by addition of rapidly available calcium can modify the setting time of the cement.

Despite the numerous publications, further research is required to understand the basic properties of the cements such as setting time, fracture mechanics like effect of microporosity and fatigue properties; and cohesion.

A new milling method of synthesis for amorphous calcium phosphates have recently been proposed[23,24] which can more effectively control setting reactions and thus can support better drug delivery systems. Moreover such synthesized CPCs can further be used to synthesise calcium phosphate granules and porous blocks having very high surface areas that are biologically very reactive and can be potent drug carriers. Still there remains a large gap between cement development and its clinical use.

Cement developing engineers or CPC researchers are more interested in improving the performance of CPC by studying and modifying its chemical and physical properties. Whereas clinicians are interested in a CPC that 'works', regardless of the composition. A 'working' cement should have low price, easy and reliable mixing and delivery, good visualisation during injection (e.g. for vertebroplasty) and faster replacement with bone or rapid bone apposition.

CPC has poor injectability compared to PMMA cements. Recent research of cement injectability have enabled a better understanding of CPC injectability[25] and have also provided innovative solutions[26,27] such as cheaper alternatives to the use of rheological agents such as sodium hyaluronate or chondroitin sulphate.

Earlier works showed that brushite CPC had a faster rate of resorption than apatite CPC[22]. However, in vivo transformation of brushite into apatite[28], impairs its resorption rate. This transformation can be delayed by the addition of a soluble magnesium salt. Apatite CPC resorption rate can also be increased by incorporation of macropores in the structure. This also results in higher volume of bone ingrowth.

The rheological behaviour of CPC is largely unknown. Temperature has a great impact on the setting properties of CPC. The setting time of apatite can be reduced by three- to four times by increasing the temperature from 20 to 37°C. Also, addition of radio-opacifiers into CPC gives better visuality during injection though solutions are not trivial. Recent and future

developments will reduce the gap between CPC research and clinical and enable better commercialisation of more varied products that possess better clinical properties and hence improve patient life quality.

Bone substitution materials are required when the natural regeneration process of bone fails to heal the acquired or congenital defects due to its critical size. These materials aid in healing process and work as a substitute for the missing bone providing mechanical and structural support to it. Calcium phosphate cements (CPCs) are used clinically as valuable bone substitution biomaterials for over 20 years[72]. Paste of Calcium phosphate salts in an aqueous solution is prepared. This paste transforms into either brushite or apatite as their final product upon setting depending on the composition of starting materials [73]. Hydroxyapatite is much similar to the natural bone mineral phase. Thus HA-forming compositions of CPCs show good biocompatibility, osteoconductivity and resorbility in vivo[74–75].

Injectability[72,76] is a major property required in case of minimal invasive applications, such as spinal applications, vertebroplasty, void filling and reinforcement of osteoporotic bone. This arises the need to study the consistency and cohesive properties. Conventional CPC powder mixed with a liquid phase causes intrinsic problems in handling as the paste needs to be prepared just before implantation and the prepared cement also starts immediately after mixing . Properties also change continuously during this working phase. The surgeon thus has very less time for preparation and application of CPC which again is affected by changes in environmental conditions resulting in degraded mechanical strength and inhomogeneity causing unexpected clinically relevant failures [77,78].

To overcome the above problems, ready-to-use cements were developed taking a single phase calcium phosphate powder or a mixture of several calcium phosphate powder of both brushite and apatite phases[79]. Ready-to-use cements are prepared by stabilizing the different calcium phosphate reactants with separate liquid or pasty components. In this setting reactions of the cement is initiated by the component that contains an aqueous liquid. The preparation process is faster than conventional CPCs as two liquid phases can be mixed more homogeneously than powder with liquid. In this dual chamber syringes equipped with a mixing device are used which has a few advantages i.e., reduced time requirement for paste preparation, less risk of contamination, enhanced reproducibility and immediate injection of the mixture in the defective tissue. Ready-to-use cements can also be prepared by the use of water-miscible non-aqueous liquids like glycerine [80] or polyethylene glycol (PEG)[81]. Miscibility with water and biocompatibility of the components are important for such systems [78]. In such cases pre-

mixed paste are prepared under defined conditions and can be stored until use. This is because powder and liquid are not in contact and are mixed during surgery thus retaining the adjusted viscosity and provides longer injection time for the surgeon as the cement setting starts when it is delivered to the aqueous environment of the defect site [81–83]. The α-TCP based CPC formulation of Biocement D[84] was used as principal cement component for the development of ready to use cement formulation. The paste-CPC was injectable, had better cohesion properties, better compressive strength and longer shelf life compared to conventional pl-CPC of similar cement composition. The final product formed from the setting reaction was found to be nanocrystalline HA as in powder/liquid CPC. This concept provides a choice to mix the CPC paste with aqueous components that may contain drugs or biological components or autologous biological fluids like blood or bone marrow aspirate. Thus ready-to-use paste-CPC opens new opportunities for simplified filling of bone defects and augmentation of osteosynthetic defects.

As discussed earlier that despite its favourable bone bonding properties, calcium phosphates exhibits poor mechanical strength restricting its use in non-load bearing defects[30] or in pure compression loading[29]. Enhancement of the mechanical properties can be achieved by forming CPC composite materials either with polymers that interpenetrate the porous matrix or with fibrous reinforcements[33] thus extending the applicability of calcium phosphates[33].

Calcium phosphate composites formed by addition of polymers have been extensively reviewed by several authors (e.g. [30,34,35]).Reinforcement of brittle CPC with fibers[33] particularly toughening by long continuous fibers[37] is one of the most successful approaches in enhancing its mechanical behavior. Brittle materials suffer a sudden failure without any significant preceding plastic deformation. Thus work needed to induce failure and fracture deformation is less.

Macroscopic behaviour of FRCPC is a combined result of strength and stiffness of both fiber and cementitious matrix, matrix toughness, mechanical interaction between fibers and matrix and supplementary effects of polymeric additives or aggregates. Apart from material strength, materials show different behavior in case of two aspects (i) strain to failure (ii) the area below the curves which is a measure of energy necessary to yield material failure. Both properties increase in the order of their failure modes i.e., brittle < tension softening (quasi-brittle materials) < strain hardening (pseudo strain hardening; highly ductile materials).

In case of pure brittle failure immediate fracture occurs in which ahigh amount of energy is absorbed due to fracture of fiber reinforced cementitious composites. The strain to failure is

also higher in this case. Quasi-brittle composites show tension softening behavior. Here fiber bridging in the single crack opening dissipates fracture energy and delays the fracture into pieces. Peak load here results in fracture of the matrix thus the composite fails to carry any substantial load further when peak load is reached. Most desirable behavior for fiber reinforced composites i.e., ductility can be achieved with (pseudo) strain-hardening behavior. It can bear even more load after initial matrix cracking. Here a network of multiple cracks is formed thus dissipating more energy in the fracture process[38,39]. Fibers influence the fracture processes ahead of a crack tip in the frontal process zone (FPZ)[40], behind the crack tip in the crack bridging wake[41] and adjacent to the crack plane[42].

In a monolithic CPC, catastrophic failure occurs due to fast crack propagation from the initial crack[33]. In fiber reinforced composites depicting quasi-brittle behavior, the linear elastic region ends with the first cracking of the matrix. Behaviour of the FPZ determines the first crack strength of the composite[40]. The material softens as the crack propagates. High ductility of the fiber reinforced cement results in its high bending capacity. It is a frequently observed trend in FRCPC research[31,36,45,48] that strength and ductility of the composites increases with fiber content. Injectability of CPC is required for its accessibility onto narrow cavities [46,47].

Viscosities of short fiber reinforced CPC is low enabling needle injection of the fiber-cement paste. Such composites are claimed to be isotropic in their properties[49]. The use of woven fibers[31,43,44] has been less common and provides anisotropic reinforcement. Fiber reinforced calcium phosphate cements (FRCPC) area newly developed class of biomedical materials in which hydroxyapatite is generally reinforced with different fiber materials to overcome the brittle fracture behavior of conventional CPC by ductilization. The reported increase in toughness (work of fracture) between FRCPC and CPC reaches two orders of magnitude.

In the present work we have studied the reinforcement of Silicon doped alpha tricalcium phosphate by addition of e-glass fiber. E-Glass fiber is a bioinert material. Though silicon addition suppose to increase the bioactivity but it decreases the mechanical strength of the cement. Without compromising the bioactivity of silicon, our objective is to enhance the mechanical strength of Si doped α-TCP by addition of 10 weight% e-glass fiber which can arrest the crack, deflect the crack and increase the fracture toughness as well as mechanical strength.

Objective:: To study the effect of Si doping and e-glass fiber addition on physicochemical and mechanical properties of calcium phosphate cement.

3. EXPERIMENTAL PROCEDURE

3.1 Materials

3.1.1 $CaCO_3$ as Calcium source

Molecular weight = 100gm

Density = 2.71 g/cm³

Company = Vico Trading and Production Co., Ltd - Vietnam

3.1.2 $CaHPO_4$ as Phosphorus source

Molecular weight = 136.06gm

Density = 2.93 g/cm³

Company = Sigma-Aldrich Co., India

3.1.3 TEOS as Silicon source

Molecular weight = 208.33gm

Density = 940.00 kg/m³

Company = TEOS Powertrain Engineering, Trappes

3.1.4 HNa_2O_4P for preparation of cement liquid

Molecular weight = 141.98gm

Density = 1.679 g/cm³

Company = Shanghai Orgpharma Chemical Co.,Ltd, China

3.1.5 e-glass fiber for reinforcement

Tensile strength[96] = 3445 MPa

Compressive strength = 1080 MPa

Density = 2.58 g/cm^3

Thermal expansion = 5.4 μm/m.°C

Softening temperature = 846°C

3.1.6 Reagents for ion concerntration in SBF

NaCl(99.5%)as source of Na^+ and Cl^-

Molecular weight = 58.44 g/mol

Density = 2.16 g/cm³

Company = Merck, Germany

$NaHCO_3$(99.5%) as source of HCO_3^-

Molecular weight = 84.007 g/mol

Density = 2.20 g/cm³

Company = Merck, Germany

KCl(99.0%) as source of $K^+$and Cl^-

Molecular weight = 74.5513 g/mol

Density = 1.98 g/cm³

Company = Merck, Germany

$MgCl_2.6H_2O$(99.0%)as source of Mg^{2+}

Molecular weight = 95.211 g/mol

Density = 2.32 g/cm³

Company = Merck, Germany

$CaCl_2.2H_2O$(99.0%)as source of Ca^{2+}

Molecular weight = 147.0146 g/mol

Density = 1.85 g/cm^3

Company = Merck, Germany

$Na_2HPO_4.2H_2O$(99.5%)as source of HPO_4^{2-}

Molecular weight = 301.9330 g/mol

Density = 1.7 g/cm3

Company = Merck, Germany

Na_2SO_4 as source of SO_4^{2-}

Molecular weight = 142.04 g/mol

Density = 2.66 g/cm³

Company = Merck, Germany

$(CH_2OH)_3CNH_2$ as buffer

Molecular weight = 121.1356 g/mol

Density = 0.84 g/ml

Company = Angus Chemical Company, Buffalo Grove, IL, US

HCl (37 vol%) for H^+ ion

Molecular weight = 36.5 g/mol

Density = 1.48 g/ml

Company = Carlo-Erba, Rome, Italy

3.2 Methods

3.2.1 Synthesis of Alpha Tricalcium Phosphate

At first hydrated di-calcium phosphate (DCPD having molecular mass=172.09gm) was heated at 180^0C for 4hours to convert it to DCPA. Then calcium hydrogen phosphate ($CaHPO_4$having molecular mass=136.06gm) and calcium carbonate($CaCO_3$ having molecular mass=100gm) were taken in the molar ratio of 2:1 ie.,27.2gm of di-calcium phosphate anhydrous (DCPA) and 10gm of $CaCO_3$. Then the required amounts of DCPA and $CaCO_3$ were ball milled in iso-1-propanol for 12 hours for uniform mixing. After milling the sample was collected and dried. The dried sample was then crushed to powder and was sintered at 1300°C for a period of 4 hours. This was followed by rapid air quenching of the samples. After sintering the sample was ground and sieved to obtain a particle size of 38μm.

3.2.2 Synthesis of 1mol% Silicon doped Alpha Tricalcium Phosphate

In this process tri-ethyl orthosilicate solution (TEOS having molecular mass=208.33gm) was taken for silicon doping of Alpha Tricalcium Phosphate.Calcium hydrogen phosphate ($CaHPO_4$having molecular mass=136.06gm) and calcium carbonate ($CaCO_3$ having molecular mass=100gm) were taken in the molar ratio of 2:1 ie.,27.2gm of di-calcium phosphate anhydrous (DCPA) and 10gm of $CaCO_3$for the preparation of Si doped α-TCP. Di-calcium

phosphate (DCPD having molecular mass=172.09gm) was heated at 180^0C for 4hours to convert it to DCPA. About 12drops of TEOS (0.1166gm=0.1249ml)was then added to the mixture of DCPA and $CaCO_3$. Then ball milling of the required amounts of DCPA and $CaCO_3$ were done in iso-1-propanol for 12 hours for uniform mixing. After milling the sample was collected and dried. The dried sample was then crushed to powder and then sintering was done firstly at 1100°C for 2 hours and then at 1300°C for a period of 4 hours. This was followed by Rapid Air quenching of the sample. This was followed by ball milling of the quenched sample for 24 hours to obtain a particle size below 25 μm.

3.2.3 Preparation of anhydrous sodium hydrogen phosphate solution

Required amount of HNa_2O_4P (having formula mass= 141.98 and density= 1.7gm/cc) were mixed with distilled water to prepare 2vol% and 5vol% solutions of sodium hydrogen phosphate.

3.2.4 Preparation of SBF solution

Simulated Body Fluid was prepared by the standard Kokubo-Method.

Table1: Chemical composition of SBF solutions[97]

Order	Reagent	Amount (gpl)
1	NaCl	6.547
2	$NaHCO_3$	2.268
3	KCl	0.373
4	$Na_2HPO_4.2H_2O$	0.178
5	$MgCl_2.6H_2O$	0.305
6	$CaCl_2.2H_2O$	0.368
7	Na_2SO_4	0.071
8	$(CH_2OH)_3CNH_2$	6.057
9	HCl	40mL

3.2.5 Study of hardening and setting of CPC from α-TCP and Si doped α-TCP powders

Weighed amounts of TCP powder and measured amount of sodium hydrogen phosphate solution were taken. The Powder to liquid ratio is predetermined and was maintained at 3:1.After that the calcium orthophosphate powder(s) and the sodium hydrogen phosphate

solution were mixed together to form a viscous and moldable paste that sets into a firm mass within a few minutes. When the paste becomes sufficiently stiff, it was introduced into the mould. The paste inside the moulds was then allowed to set in a 100% humid environment by covering it with wet tissue papers inside the incubator. After about 3hours of setting the set samples were removed from the moulds and immersed in SBF solution prepared by procedure 3.2.4.

3.2.6 Study of hardening and setting of 10 wt% E-glass fiber reinforced CPC

E-glass fibers were immersed in SBF solution for about 6-7 days so that the fibers gets coated with hydroxyapatite. Hydroxyapatite coating on the e-glass fiber helps in better bonding of the fibers and calcium phosphate powder after setting as it provides better entanglement of the hydroxyapatite particles formed on CPC to that of the hydroxyapatite coating on the e-glass fiber. Then a batch of Si doped α-TCP powder and glass fiber was prepared by proper and uniform mixing of e-glass fiber (10% by weight of powder taken) and Si doped α-TCP powder. The batch thus prepared was then made to set according to the process followed in procedure 3.2.5

4. CHARACTERIZATION

4.1 XRD Analysis::

X-ray diffraction analysis of the α-Tricalcium phosphate and Si doped α-Tricalcium phosphate powders and the fully set cements at periodic time intervals were performed by using Rigaku Ultima IV operated at 40KV voltage and 40 mA current at room temperature. Data were collected by using solid state detector Cu as target with scanning range of 2θ=10°–70°,and a 2θ step size of 5°/min.

The reference number of the compound formed was found from the literature and matched using PCPDF WIN program. XRD analysis of the powder samples was performed using the X'PERT HIGH SCORE software.

4.2 Hardening and Setting::

Self-setting calcium orthophosphate formulations were formed by mixing amorphous and/or crystalline calcium orthophosphate powder(s) with a 5 vol% aqueous solution of sodium hydrogen phosphate. After the calcium orthophosphate powder(s) and the solution are mixed together, a viscous and mouldable paste is formed that sets to a firm mass within a few minutes. E-Glass fibers were also added wherever applicable. Then the set cement samples were removed from the moulds and are immersed into the SBF solution.

Initial (I) and final (F) setting times were measured using the Vicat needle (ASTM, C191). A ring with a diameter of 20 mm and a height of 10 mm were used as mould. All measurements were performed at room temperature. The setting times were obtained from a set of three samples each of α-Tricalcium phosphate and Si doped α-Tricalcium phosphate cement.

4.3 Study of kinetics of setting reaction in CPC

Samples of set cement were removed from the mould and immersed in SBF at 37 °C for 10 days. At intervals of 0 hour, 10 hour, 1 day, 2day upto 10 days the immersed samples were removed from the SBF solution. The samples were then crushed to powder and immersed into acetone to stop the hydraulic setting reaction. Putting the samples onto acetone arrests theα-TCP to HA conversion reaction at that point. This aids in determining the conversion rate of the reaction.

After drying in air (to remove acetone), the sample pieces were milled under 70 μm, and the

X-ray diffraction patterns were obtained from 2θ = 10°-70° at a step size of 20°/minute. α-TCP exhibits a peak of 2.905 at plane (170). Hydroxyapatite has a peak of 2.814 at plane (211).

The extent of conversion of α-TCP into HA was calculated using the following equation:

$$\mathbf{Conv.= [(I_0/Is)-(I_t/I_s)]/[(I_0/I_s)-(I_\infty/I_s)]}$$

where,

I_0 = integrated intensity of the peaks corresponding to the converted phase at the beginning

I_t = integrated intensity of the peaks corresponding to the converted phase at time t

I_∞ = integrated intensity of the peaks corresponding to the converted phase at infinite time

I_s = integrated intensity of the peak corresponding to the internal standard.

4.4 Determination of Mechanical Strength:

The set CPC in moulds were immersed in SBF solution at 37.5°C. Samples were then removed from SBF at periodic time intervals for use as test specimens and compression tested in Tinius Olsen H10KS Universal Testing Machine. Load was applied at a speed of 1mm/min at room temperature until failure. Three replicas of each cement (α-TCP, 1% Si doped α-TCP without e-glass fiber, 1% Si doped α-TCP with e-glass fiber) formulations were tested.

Diametral Tensile Strength is calculated using the formula::

$$\mathbf{DTS= 2\sigma/ \Pi dt}$$

where,

σ = Load in Newton

Π = constant (3.14)

d = diameter of the samples

t = thickness of the samples

4.5 Analysis of microstructure using Scanning Electron Microscopy::

The samples were taken out from SBF after definite time interval and dried in air. Then a 3minutesurface coating of graphite was applied to the samples. Graphite coating makes the surface of the samples conductive. This aids in better examination of the surface (fractured surface). The samples were then analyzed by means of scanning electron microscopy (NOVA NANO SEM FEG operated at voltage of 310V and 90μA current) to observe its microstructure.

5. RESULTS AND DISCUSSION

5.1 Phase evaluation of synthesized powder

The x-ray diffraction patterns of undoped α-TCP and 1 mol% Si doped α-TCP powders revealed the presence of α -TCP as major phase with traces of β-TCP formed during the quenching process.

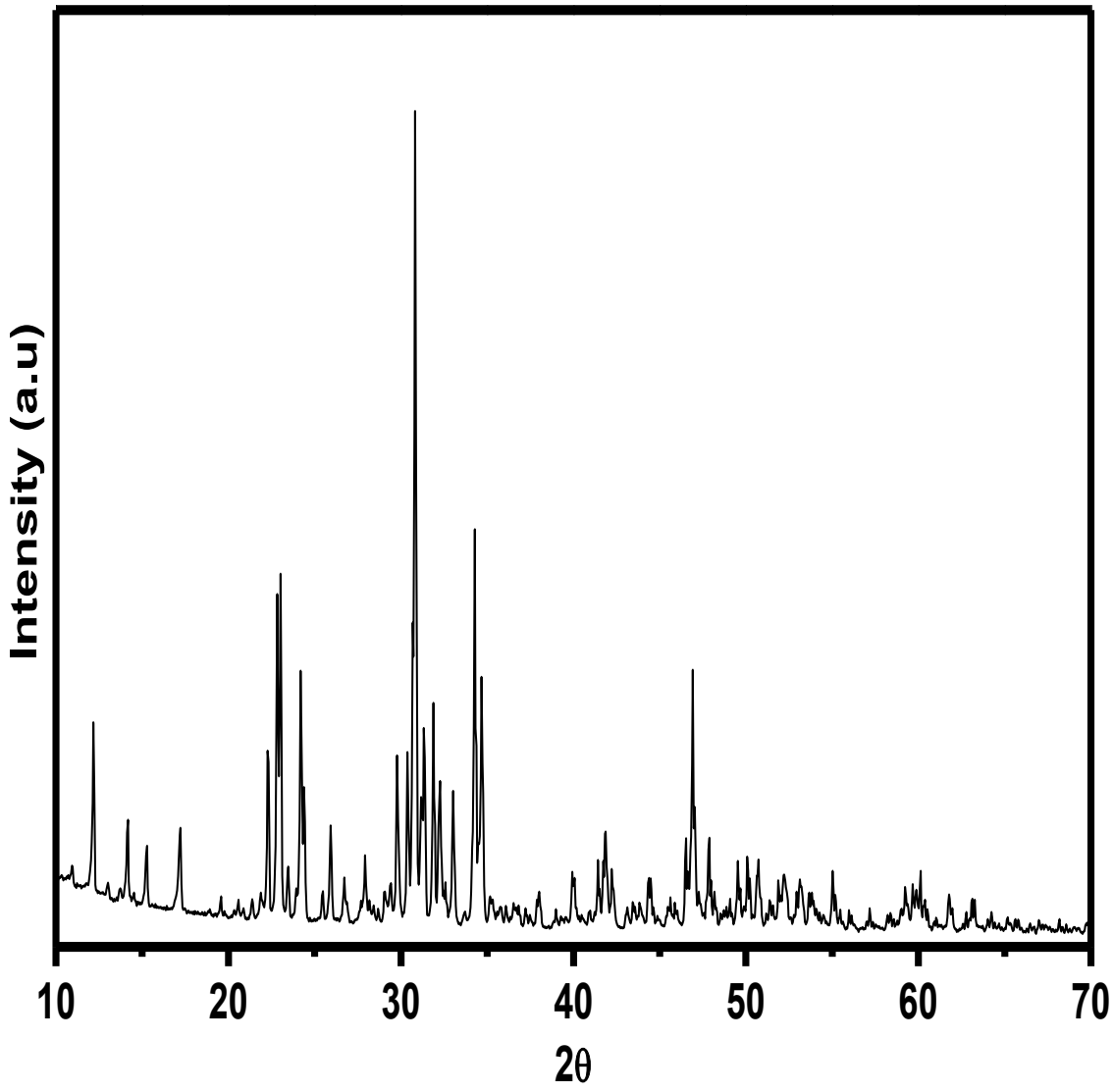

Fig.2 (a) XRD pattern of undoped α-Tricalcium Phosphate

Figure 2 (a) shows the diffraction pattern of α-TCP powder synthesized using solid state reaction of calcium carbonate and anhydrous di-calcium phosphate at 1300˚C for 6 hours. Major phase of α-TCP with trace amount of β-TCP was observed in the XRD pattern.

β-TCP was expected to transform into α-TCP beyond 1125˚C but the α-TCP phase being a metastable phase some amount of β-TCP was formed during the cooling of the powder.

Our major objective was to maximize the amount of α-TCP phase in the synthesized powder so that enhanced hydrolysis reaction of α-TCP into hydroxyapatite can occur during the setting of the cement in simulated body fluid (SBF).

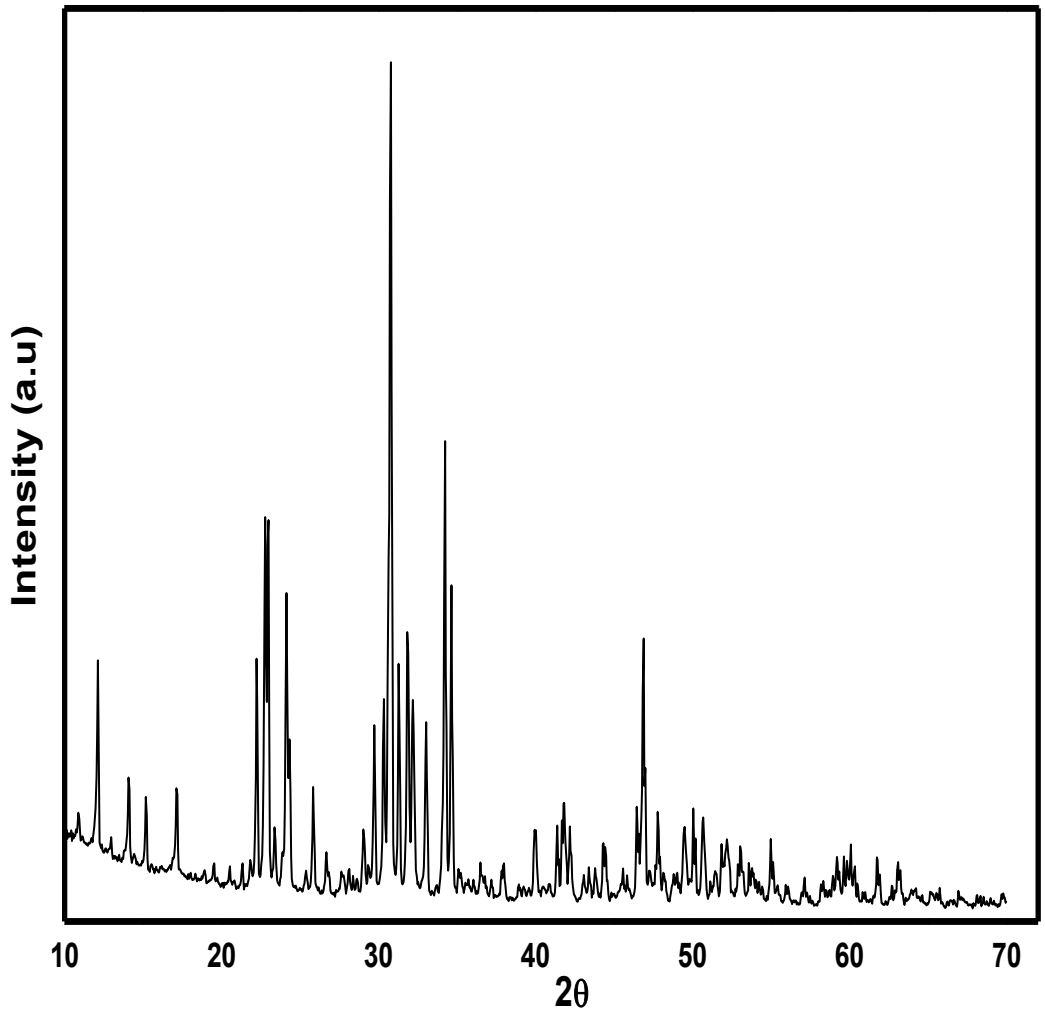

Fig.2 (b) XRD pattern of 1 mol% Si doped α-Tricalcium Phosphate

X-ray diffraction pattern of 1 mol% Si doped α-TCP powders is shown in figure 2 (b). Almost similar type of phase composition as that in undoped α-TCP was found in the XRD pattern of Si doped α-TCP.

5.2 Composition of E- glass fibre

EDAX analysis of the procured E-Glass fibre is shown in figure 3 and the elemental composition is given in table 3. The presence of Al_2O_3 in the silica glass fibre gives necessary

mechanical strength to E- glass fibre.

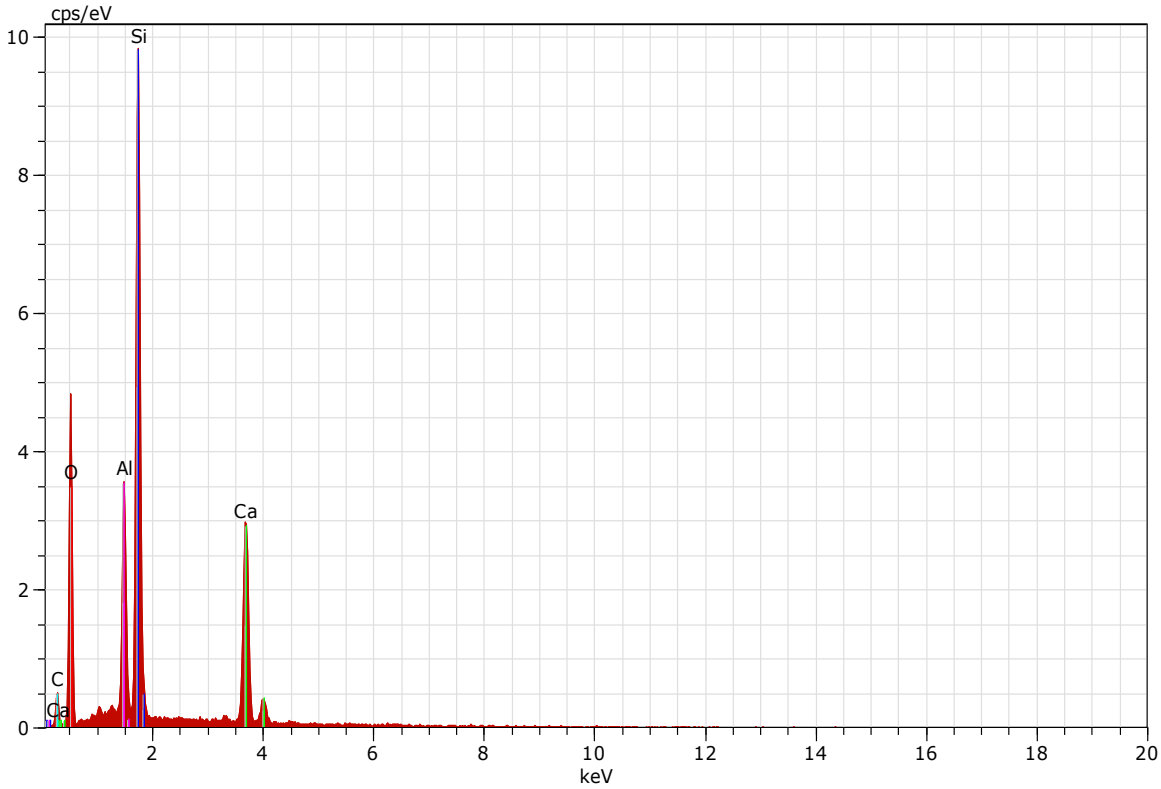

Fig. 3. EDAX analysis of E- glass fiber

Table 2: Quantitative analysis of E- glass fibre from EDAX data

Element	Atomic No.	Weight%
O	8	50.26%
Ca	20	16.2%
C	6	-
Si	14	26.04%
Al	13	7.4%

Figure 4 shows image of E-Glass fibres obtained through scanning electron microscopy. E-glass fibres having an average diameter of 14 μm and length of 1.2-1.6 mm were mixed to the extent of 10 wt% with Si-α TCP, kneaded, formulated like a paste, hardened in SBF and the physicochemical and mechanical strength of the resulted CPC were evaluated.

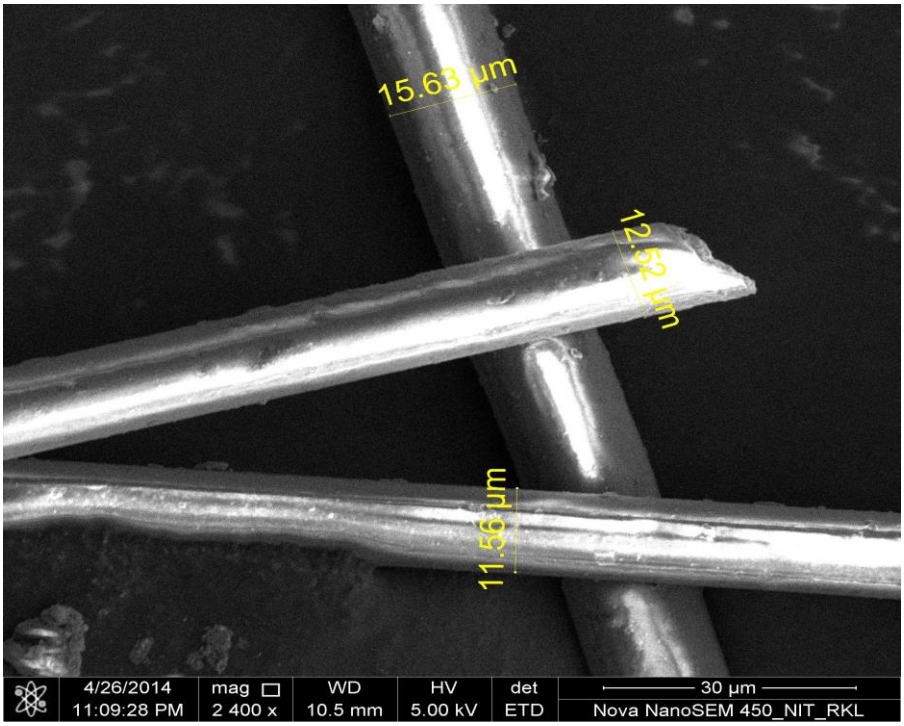

Fig.4 FESEM micrograph of E- glass fiber

5.3 Setting Time

Setting time of calcium phosphate cement was observed at a powder to liquid ratio of 1:3 using Vicat Needle. α-tricalcium phosphate cement exhibited an initial setting time of about 10 minutes and final setting time of 25 minutes, while 1% Si doped α-TCP cement showed an initial setting time of about 14 minutes and took 35 minutes for its final setting. Si doped calcium phosphate cement showed a delayed initial and final setting time as compared to undoped calcium phosphate cement because of retarded rate of hydrolysis and HA crystal growth in Si-doped α-Tri-calcium Phosphate into hydroxyapatite as compared to undoped α-Tri-calcium Phosphate. CPCs set as a result of a dissolution and precipitation process[98]. The entanglement of the precipitated crystals is responsible for cement hardening unlike acrylic bone cements, which harden due to polymerization reaction.

5.4 Kinetics of conversion into HA

The intensities of the α-TCP peaks at 22.2° (201) and 24.1° (161,-331) decreased when the time of immersion in SBF rose from 5 hr to 10 days, due to its transformation into HA. Si doped CPC showed little slower rate of conversion into HA phase as compared to undoped CPC. Si doped α–TCP cements presented a faster initial reaction than pure α-TCP but a

slower secondary reaction.

Figure 5.1 and figure 5.2 demonstrates the XRD patterns of the calcium phosphate cement derived from hydrolysis of α-TCP in SBF solutions at different time periods upto 10 days. From the XRD pattern it is evident that the increasing amount of hydroxyapatite phase is evolved due to hydrolysis of α-TCP into HA with increase in immersion time in SBF.

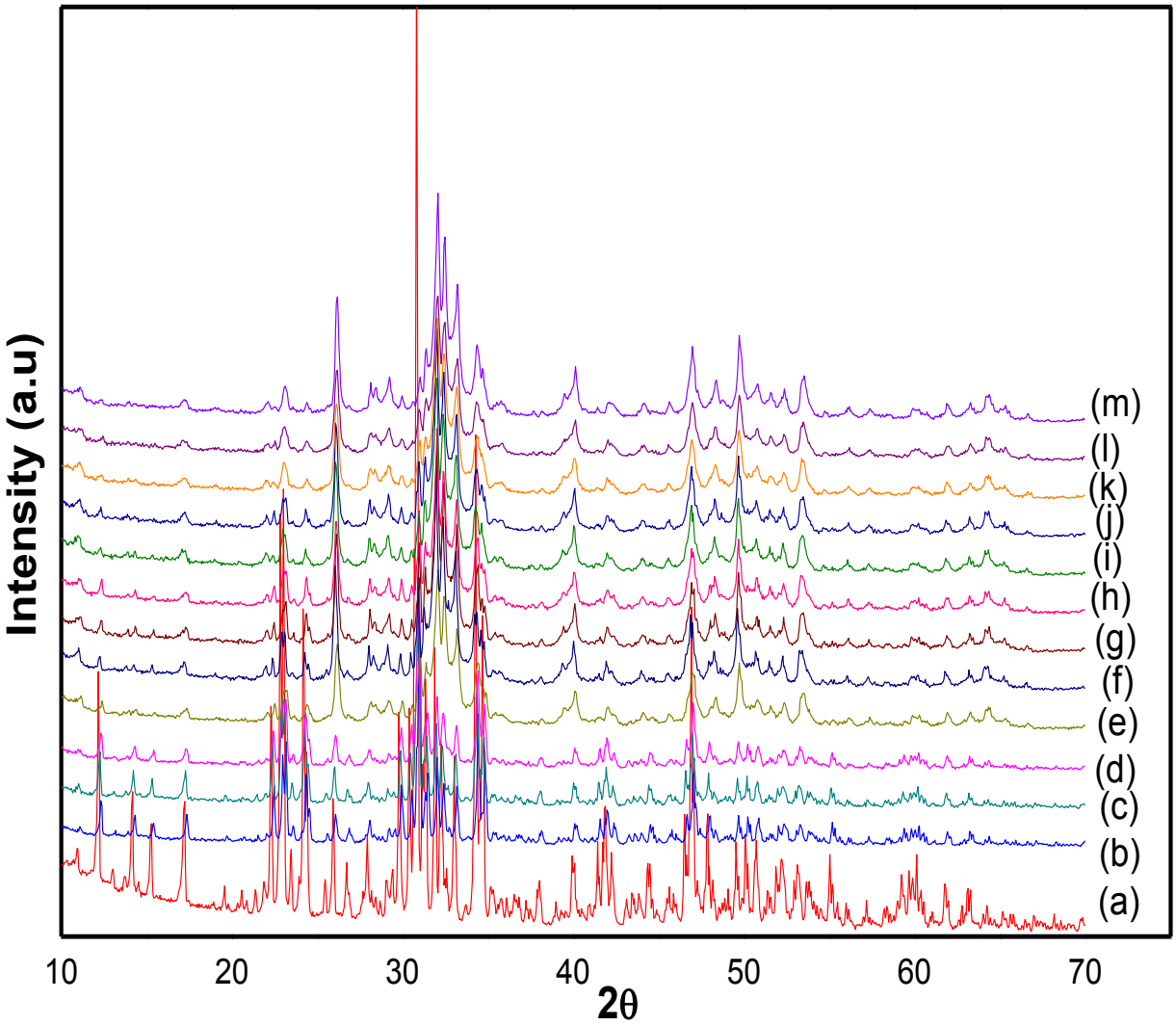

Fig. 5.1 XRD pattern of undoped CPC in SBF

XRD pattern of the α-TCP powder (a) as synthesized (b) after 0 hours of immersion in SBF (c) after 10 hours of immersion in SBF (d) after 1 day of immersion in SBF (e) after 2 days of immersion in SBF (f) after 3 days of immersion in SBF (g) after 4 days of immersion in SBF (h) after 5 days of immersion in SBF (i) after 6 days of immersion in SBF (j) after 7 days of immersion in SBF (k) after 8 days of immersion in SBF (l) after 9 days of immersion in SBF (m) after 10 days of immersion in SBF

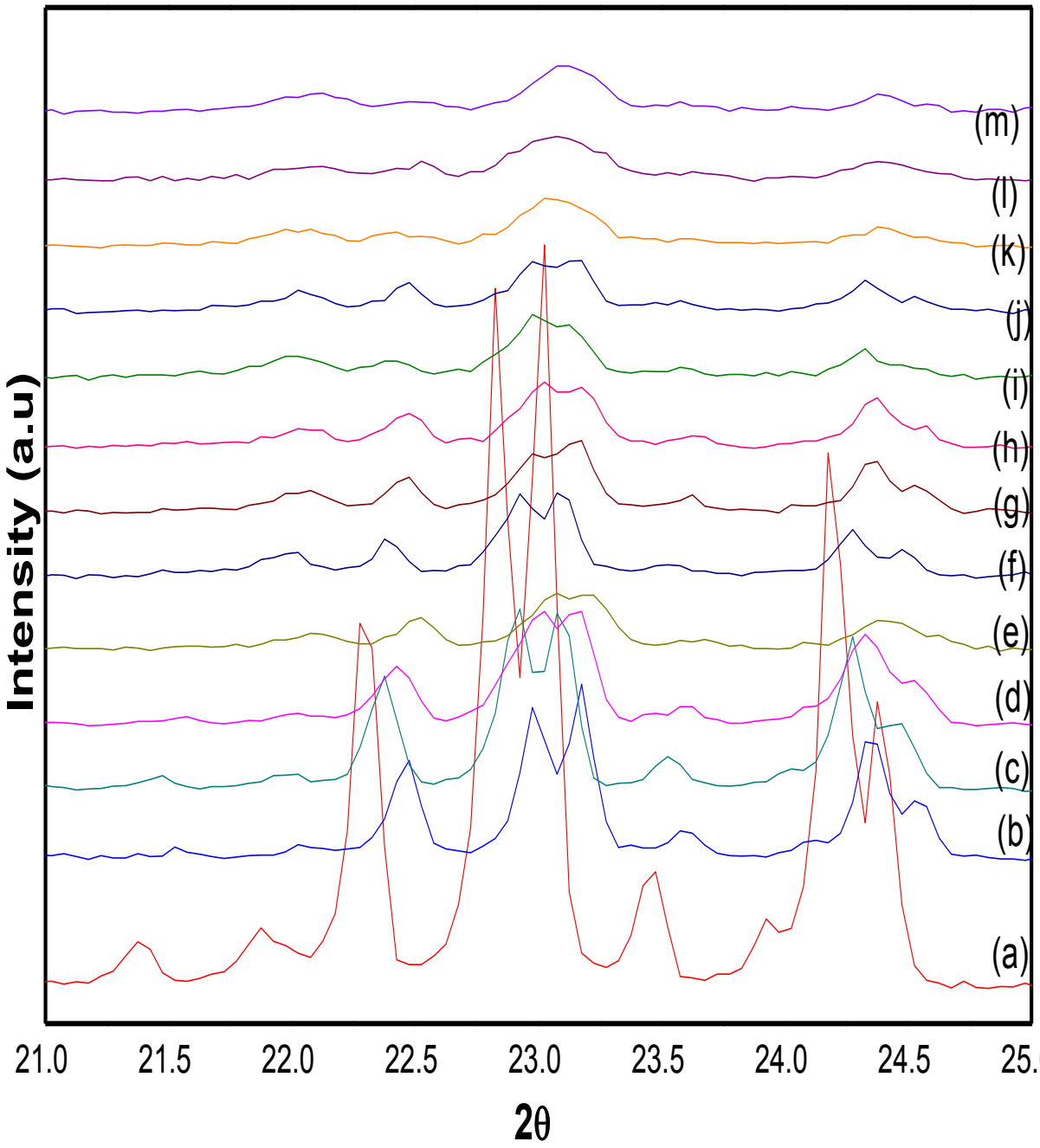

Fig. 5.2 XRD pattern of undoped CPC in SBF with 2θ varying from 21-25^{o}.

Figure 6.1 and figure 6.2 demonstrates the change in XRD patterns of 1% silicon doped of calcium phosphate cement due to hydrolysis of Si doped α-TCP in SBF solutions for different time durations. From the XRD pattern it is evident that there is an appearance of hydroxyapatite peak with disappearing α-TCP peak with increase in immersion time in SBF.

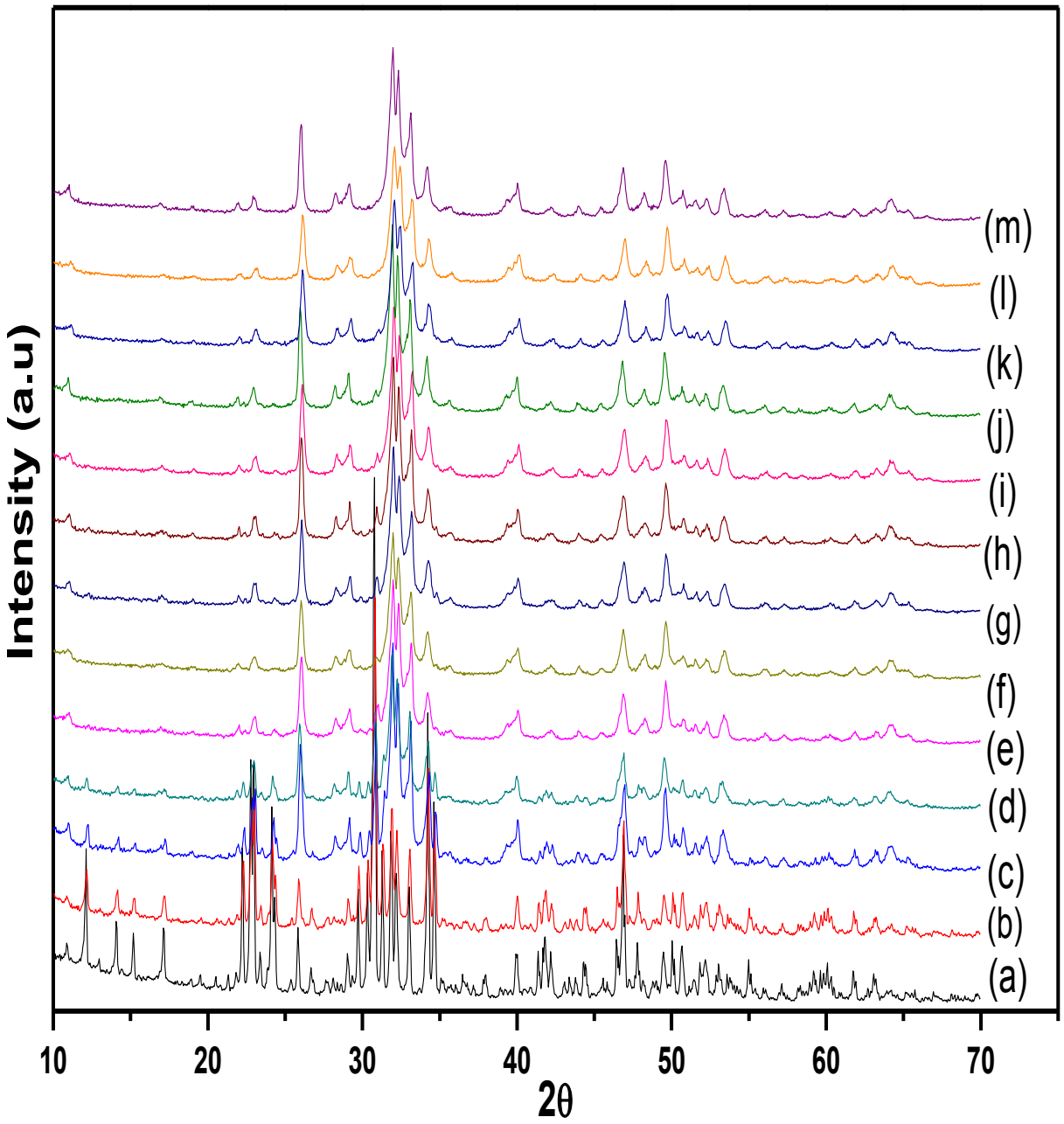

Fig. 6.1 XRD pattern of Si doped CPC in SBF

XRD pattern of the 1% Si doped α-TCP powder (a) as synthesized (b) after 0 hours of immersion in SBF (c) after 10 hours of immersion in SBF (d) after 1 day of immersion in SBF (e) after 2 days of immersion in SBF (f) after 3 days of immersion in SBF (g) after 4 days of immersion in SBF (h) after 5 days of immersion in SBF (i) after 6 days of immersion in SBF (j) after 7 days of immersion in SBF (k) after 8 days of immersion in SBF (l) after 9 days of immersion in SBF (m) after 10 days of immersion in SBF.

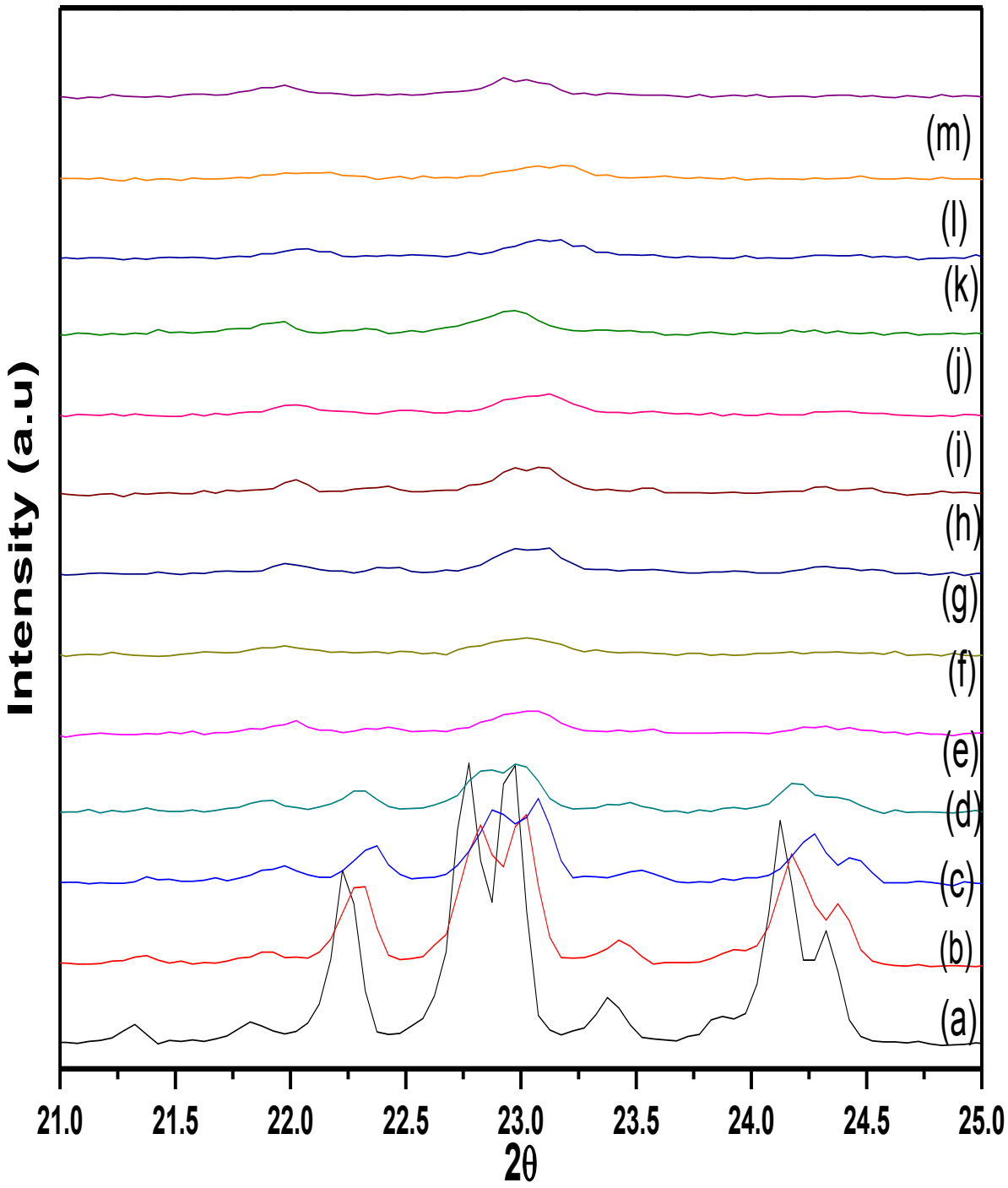

Fig. 6.2 XRD pattern of Si doped CPC in SBF with 2θ varying from 21-25^{o}.

Figure 7 shows that throughout the time period of immersion Si-doped α-TCP cement showed a slower rate of conversion into hydroxyapatite as compared to undoped α-TCP calcium phosphate cement because Silicon retards the hydrolysis of tri-calcium phosphate into hydroxyapatite.

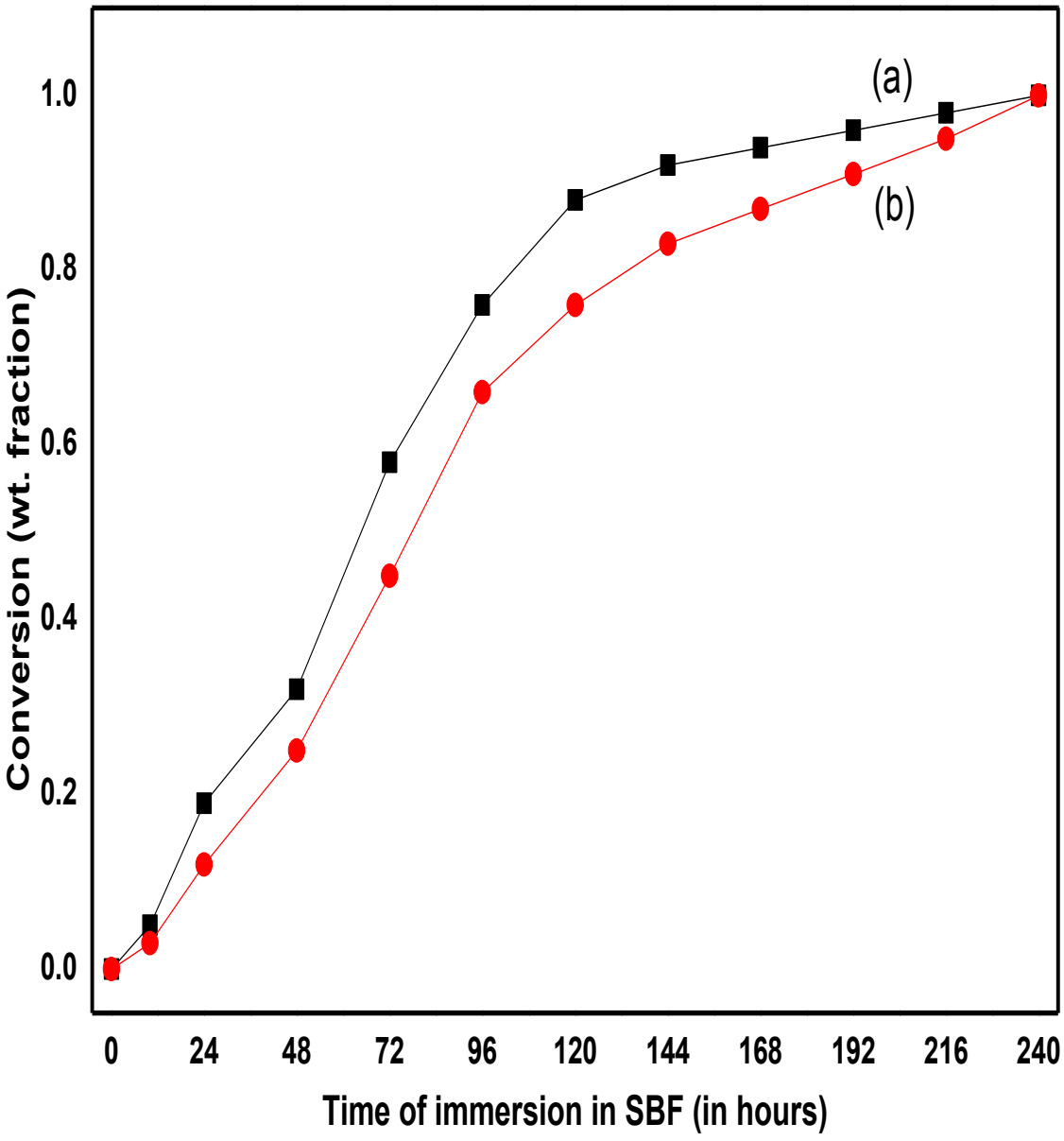

Fig. 7. Comparison of conversion rate of undoped and Si doped CPC

In figure 7, (a) is for undoped α-TCP and (b) for 1% Si doped α-TCP cement.

5.5 SEM::

Scanning Electron Microscopy examination of the fully set cements soaked in SBF for 10 days at 37°C showed the formation of a thin and dense bonelike apatite layer on their surface. The newly formed apatite layer consisted of nodules of crystal platelets grown together, forming a compact layer. The examination with SEM of the set cement showed a network of entangled plate-like apatite crystals grown from the α-TCP grains. Typical Hadley grains could be observed showing an empty thick shell of hydration products inside which a α-TCP grain had fully reacted. Si doped CPC showed thicker and denser apatite formation as

compared to undoped CPC indicating its higher bioactivity.

Figure 8 shows that the growth of flower-like hydroxyapatite crystals over the fracture surface of α-TCP derived cement upon its soaking in SBF solution. These crystals were mostly elongated. The acicular structure aids in better entanglement of crystals and formed a compact microstructure.

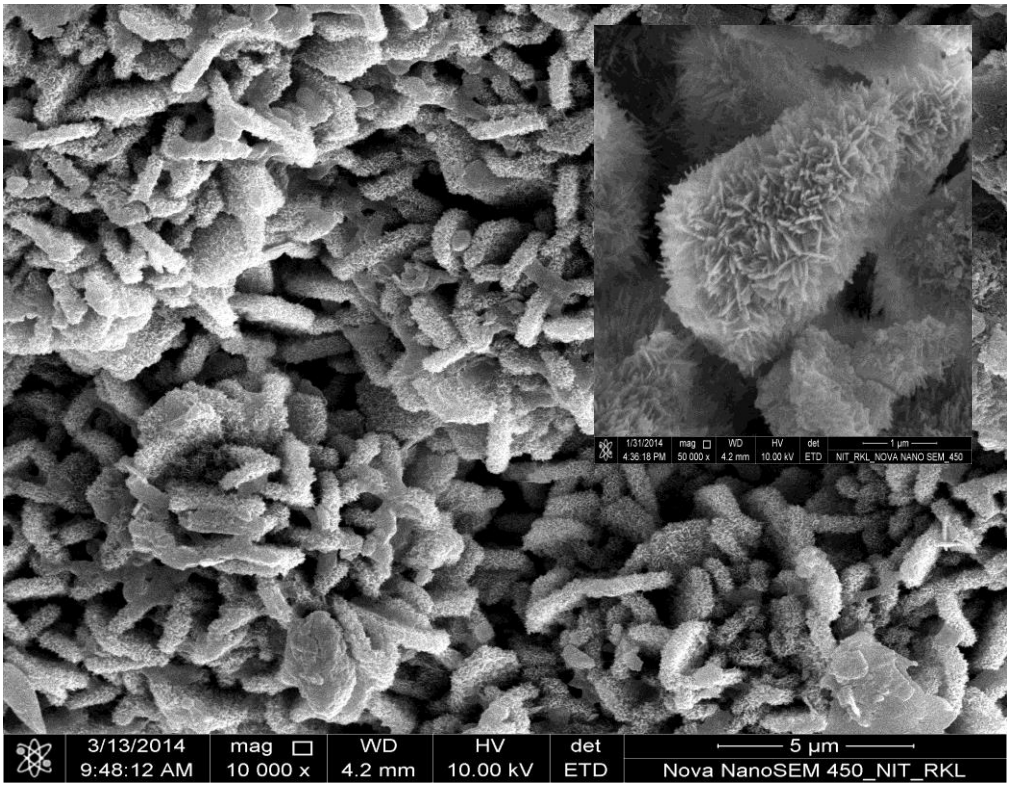

Fig. 8. FESEM micrograph of undoped CPC

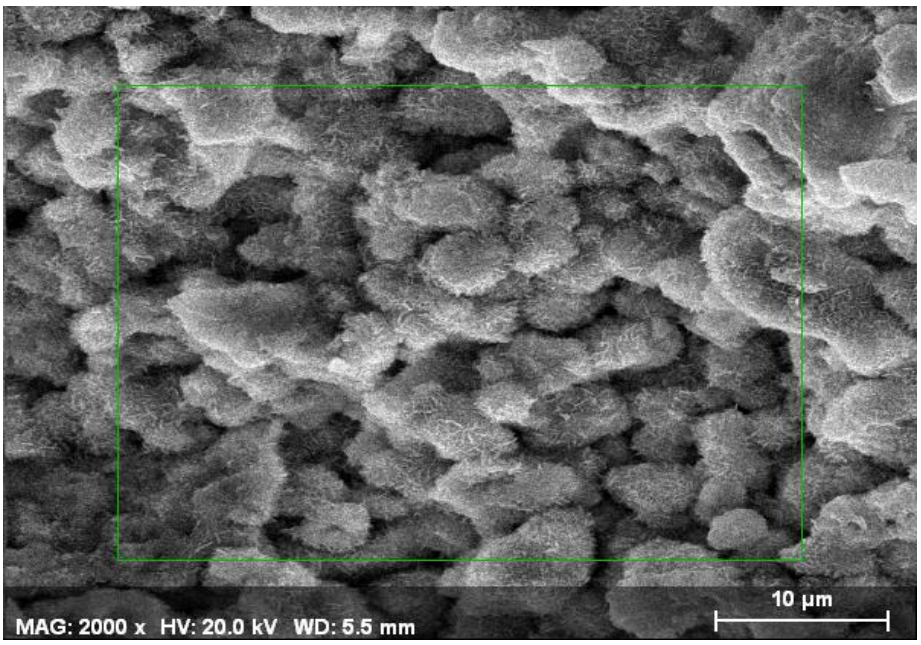

Fig. 9.1: FESEM micrograph of Si doped CPC

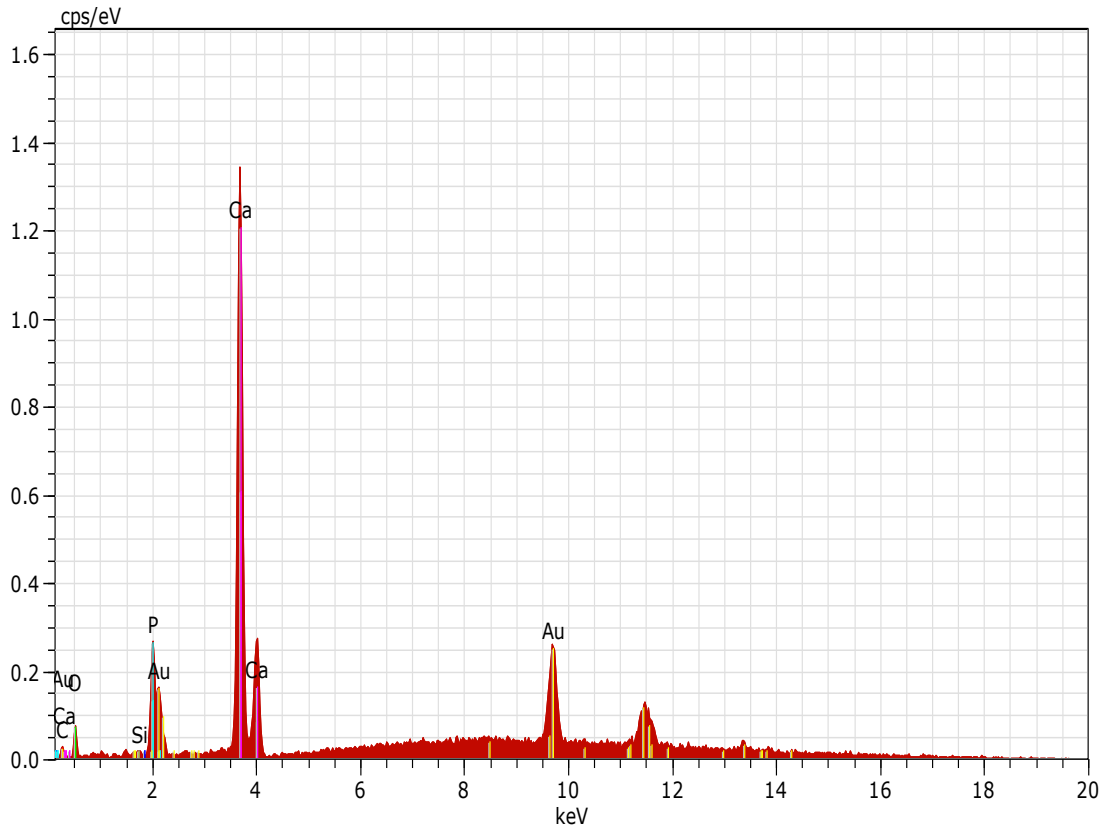

Fig. 9.2: EDAX analysis of Si doped CPC

Figure 9.1 shows the microstructure of Si doped CPC after immersion in SBF for 10 days. EDAX analysis of the part of microstructure in figure 9.1 confirming the presence of different constituent elements present in Si doped α-TCP cement is shown in figure 9.2.

Table 3: Quantitative analysis of 1 mol% Si doped CPC from EDAX data

Element	Atomic No.	Weight%
Ca	20	45.56%
P	15	26.94%
O	8	23.76%
Si	14	1.05%
C	6	2.67%

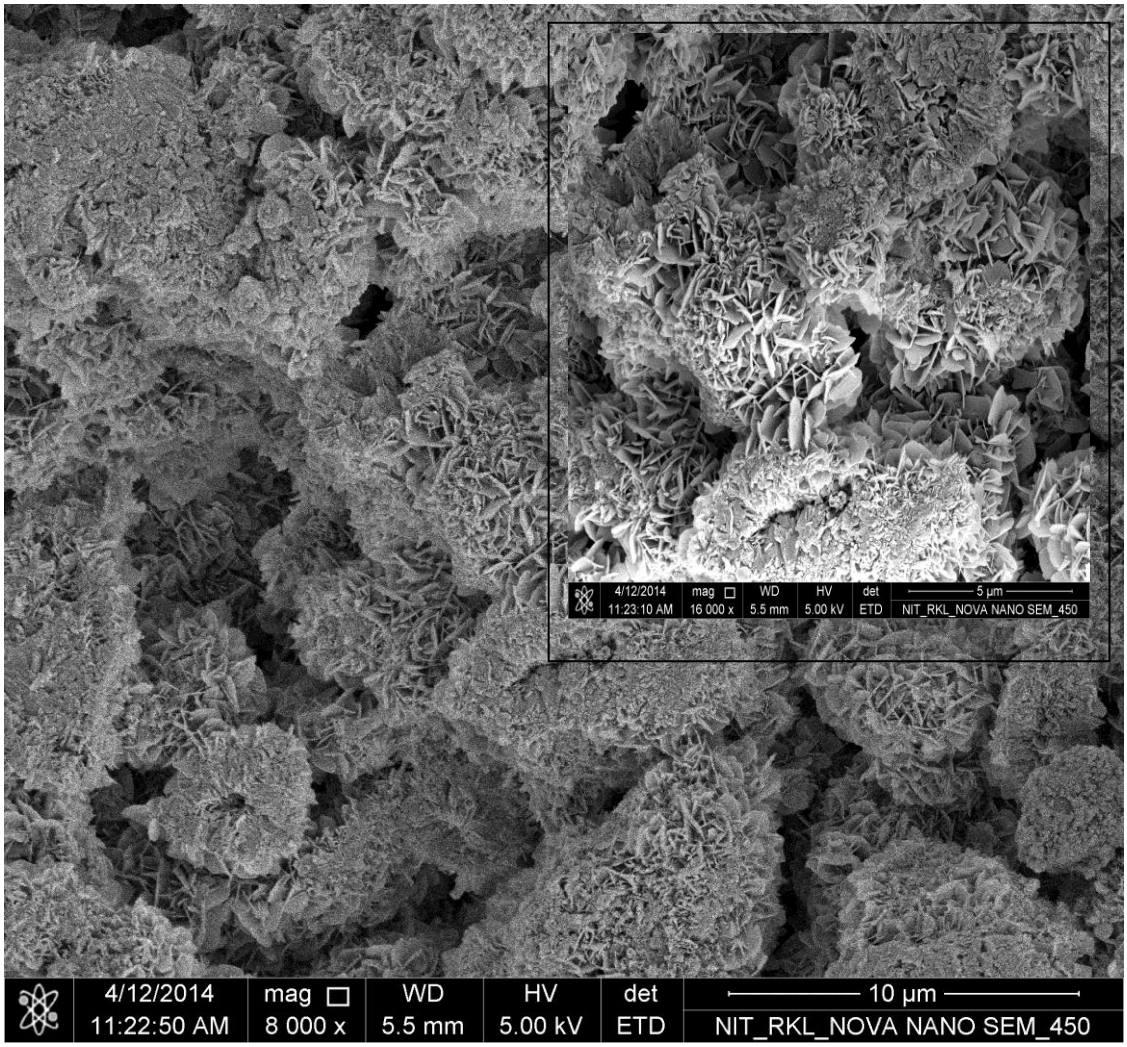

Fig. 10. FESEM micrograph of 1% Si doped CPC without e-glass fiber

Figure 10 shows the growth of spherical HA crystals in Si doped CPC. Here the hydroxyapatite crystals were larger in size and more spherical in morphology as compared to undoped CPC. More voids were present in Si doped α-TCP microstructure with lesser entanglement of hydroxyapatite crystals than undoped α-TCP cement due retarded rate of hydrolysis and slower rate of HA crystal growth in Si doped CPC.

Figure 11 shows the unmodified surface of E-Glass fiber before immersing it into SBF. It could be observed that there was no apatite growth over the surface before immersion.

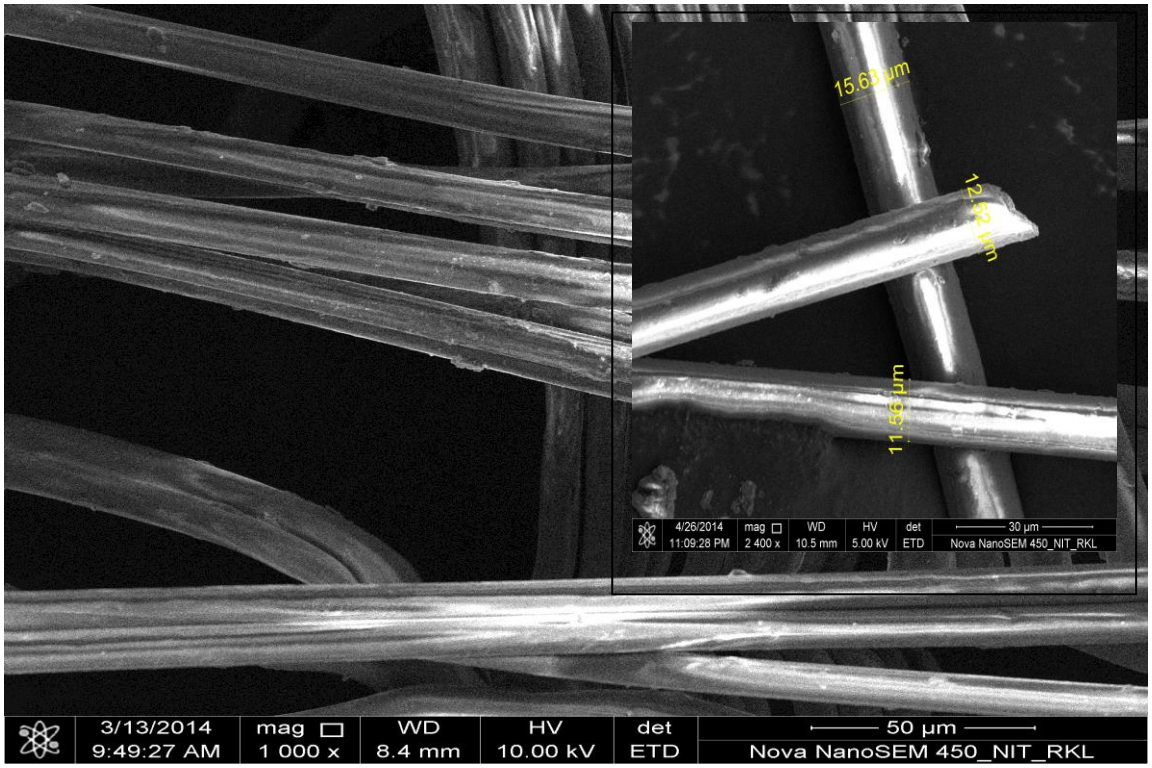

Fig. 11. FESEM Micrograph of E-Glass fiber before immersion into SBF

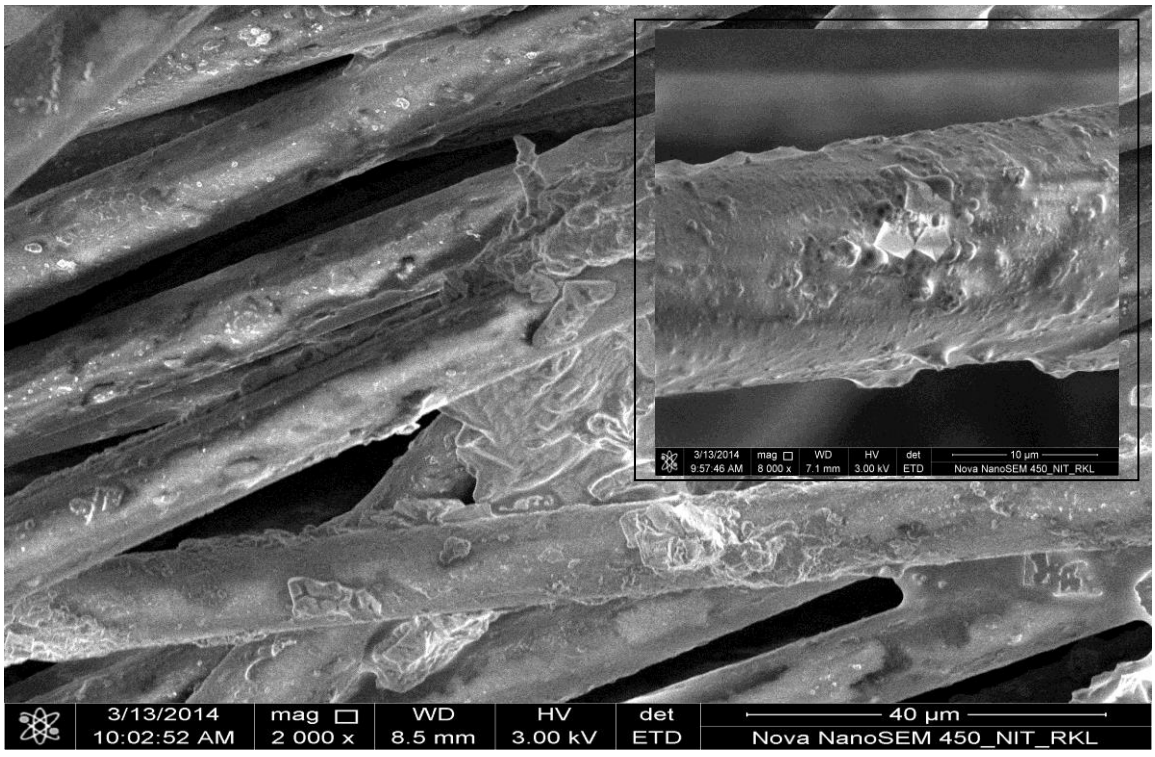

Fig. 12. FESEM Micrograph of E-Glass fiber after immersion into SBF

The SEM analysis of the e-glass fibres in figure 12 showed the growth of apatite layer over it and depicts the modified surface of E-Glass fiber caused by immersing it into the SBF

solution. A layer of apatite crystal growth could be observed over the fiber surface. The deposition of apatite layer on the surface of E-glass fibre could aid in seeding of HA crystal during setting reaction of CPC and form a better and stronger interface between glass fibres and CPC crystals.

5.5 Analysis of mechanical strength:

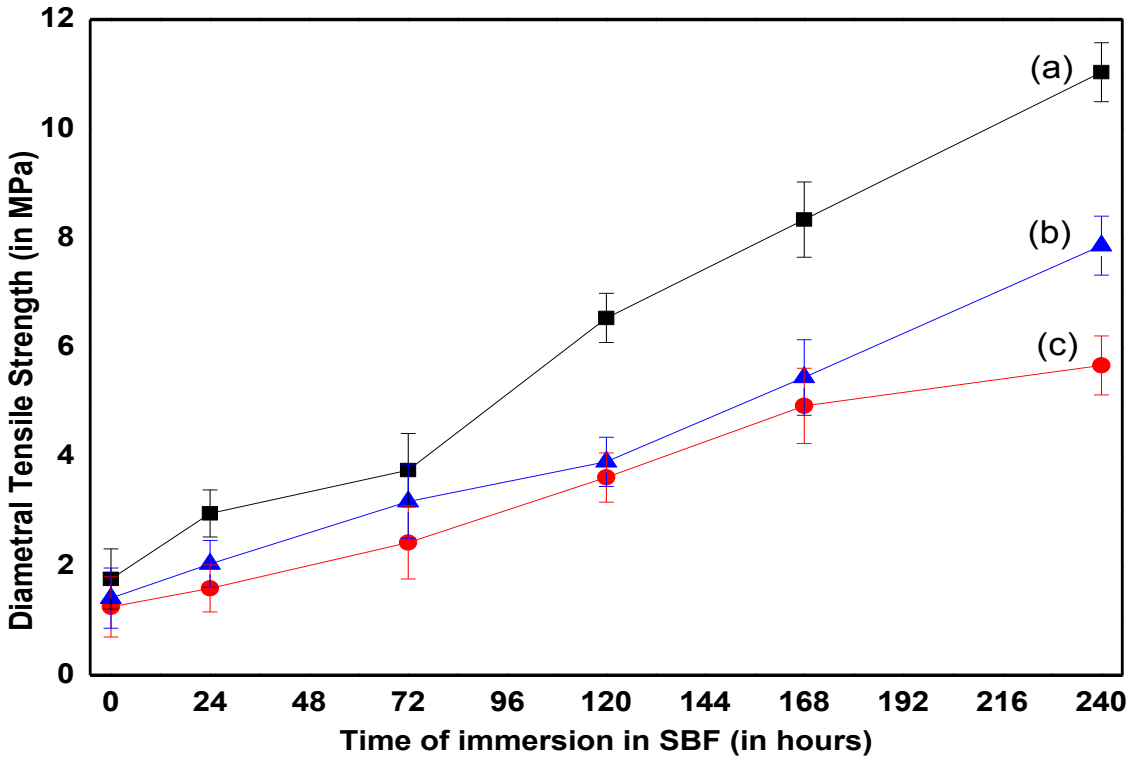

Fig. 13. Comparison of DTS of doped and undoped CPCs (a) α-TCP, (b)1% Si doped α-TCP with 10 weight% e-glass fiber, (c)1% Si doped α-TCP.

Figure 13 shows the variation of diametral tensile strength with increase in soaking time of SBF for undoped CPC, 1% Si doped CPC, and 10 weight% E-Glass fiber added Si doped CPC. In all the cases it could be seen that with increase in soaking time in SBF, DTS for CPC increased due to more amount of HA crystal growth and as a result of entanglement of HA crystals with each other to reduce porosity and compactness in the microstructure. HA crystal growth and more entanglement of crystals in the pores of the microstructure gave better compactness with increase in elapsed time in SBF. As evident from the microstructure higher amount of macro porosity and lower amount of compactness of Si-doped CPC resulted in lower DTS as compared to undoped CPC.

The plots of load vs extension of some of the CPC samples are shown below.

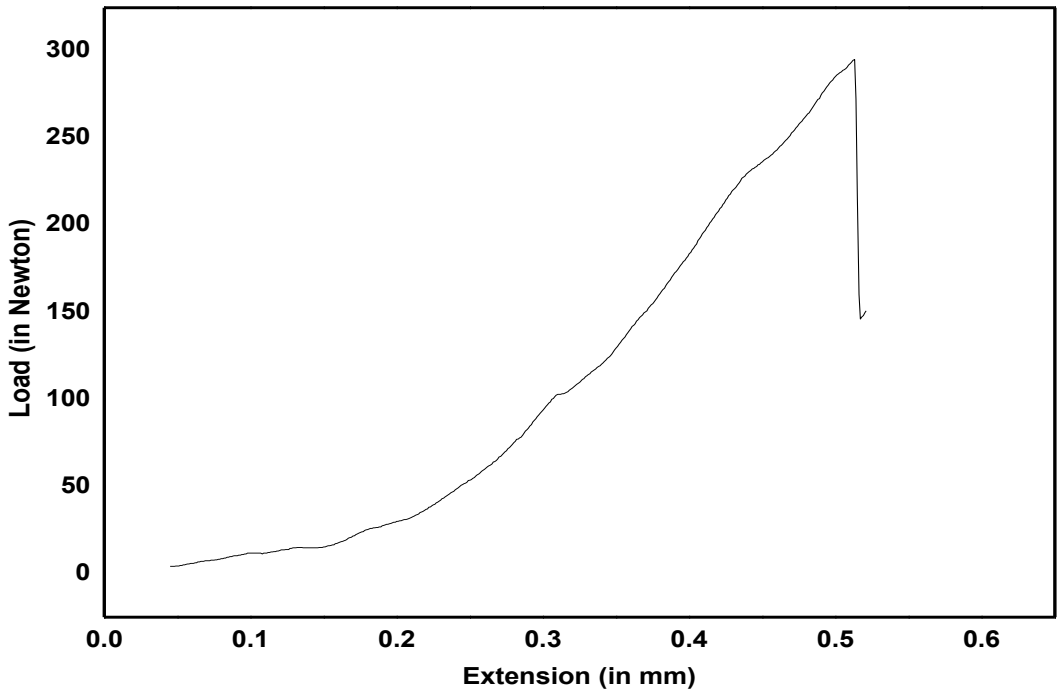

Fig. 14a: Load vs extension graph of undoped CPC

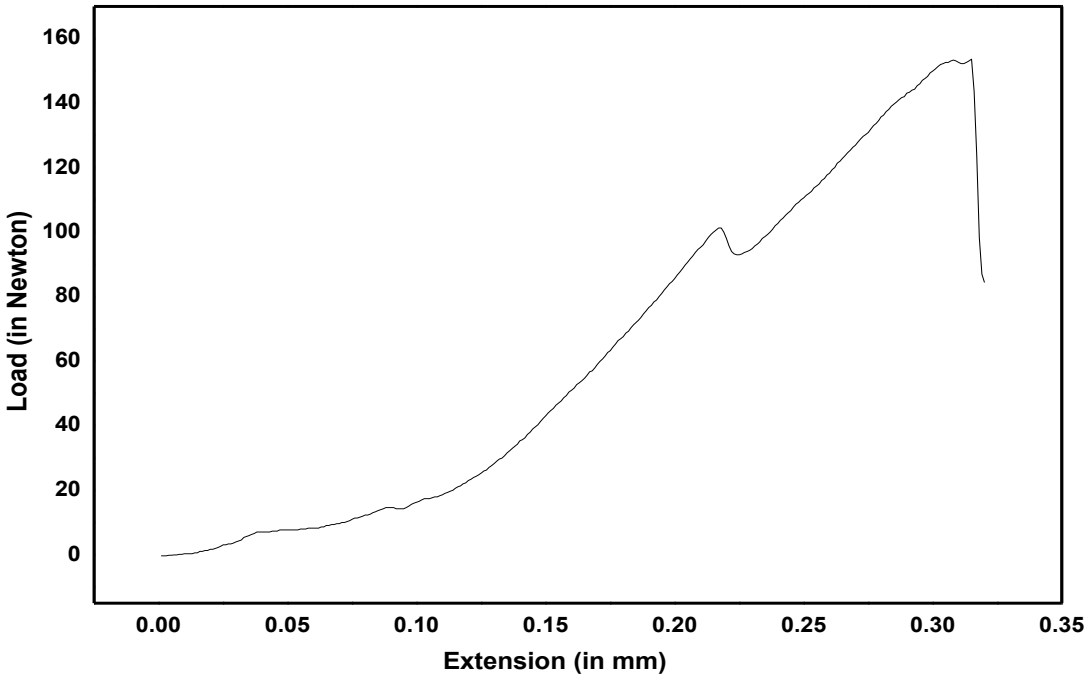

Fig. 14b: Load vs extension graph of Si doped CPC

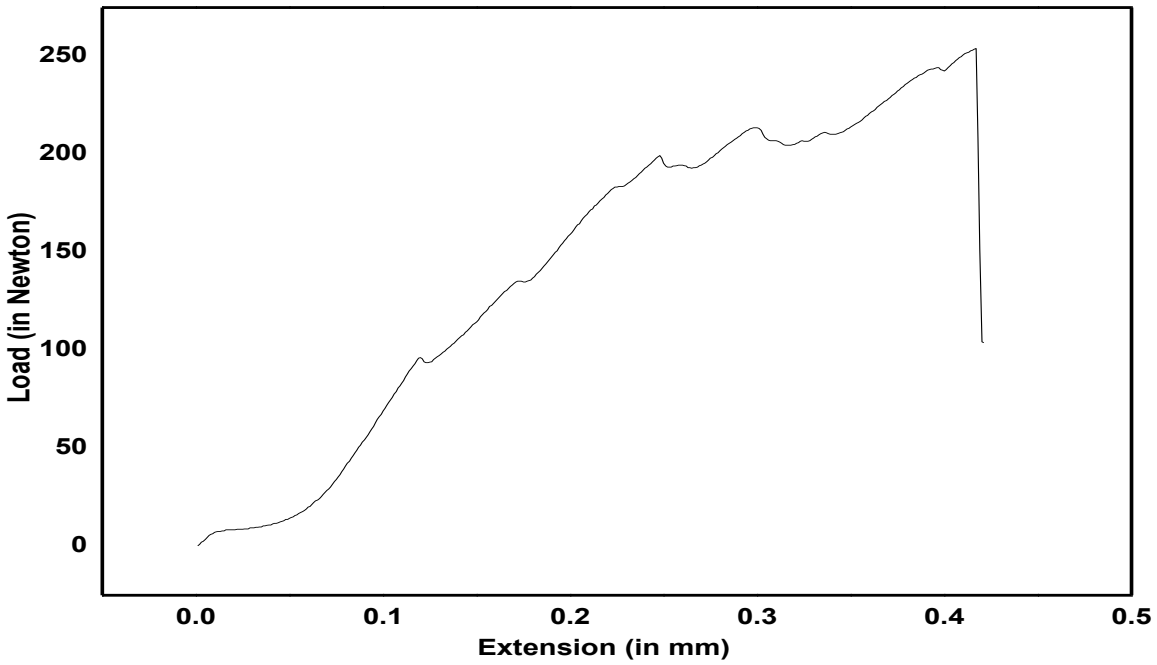

Fig. 14(c): Load vs extension graph of 10 wt% E –glass fiber reinforced Si doped CPC

Figure 14(a) shows the load vs entension graph of undoped CPC and the load vs entension graph of Si doped CPC and e-glass fiber reinforced Si doped CPC are shown in figure 14 (b) and (c) respectively. In 14(b) catastrophic failure can be observed but in figure 14(c), it could be observed that the glass fibers arrested and deflected the crack before failure.

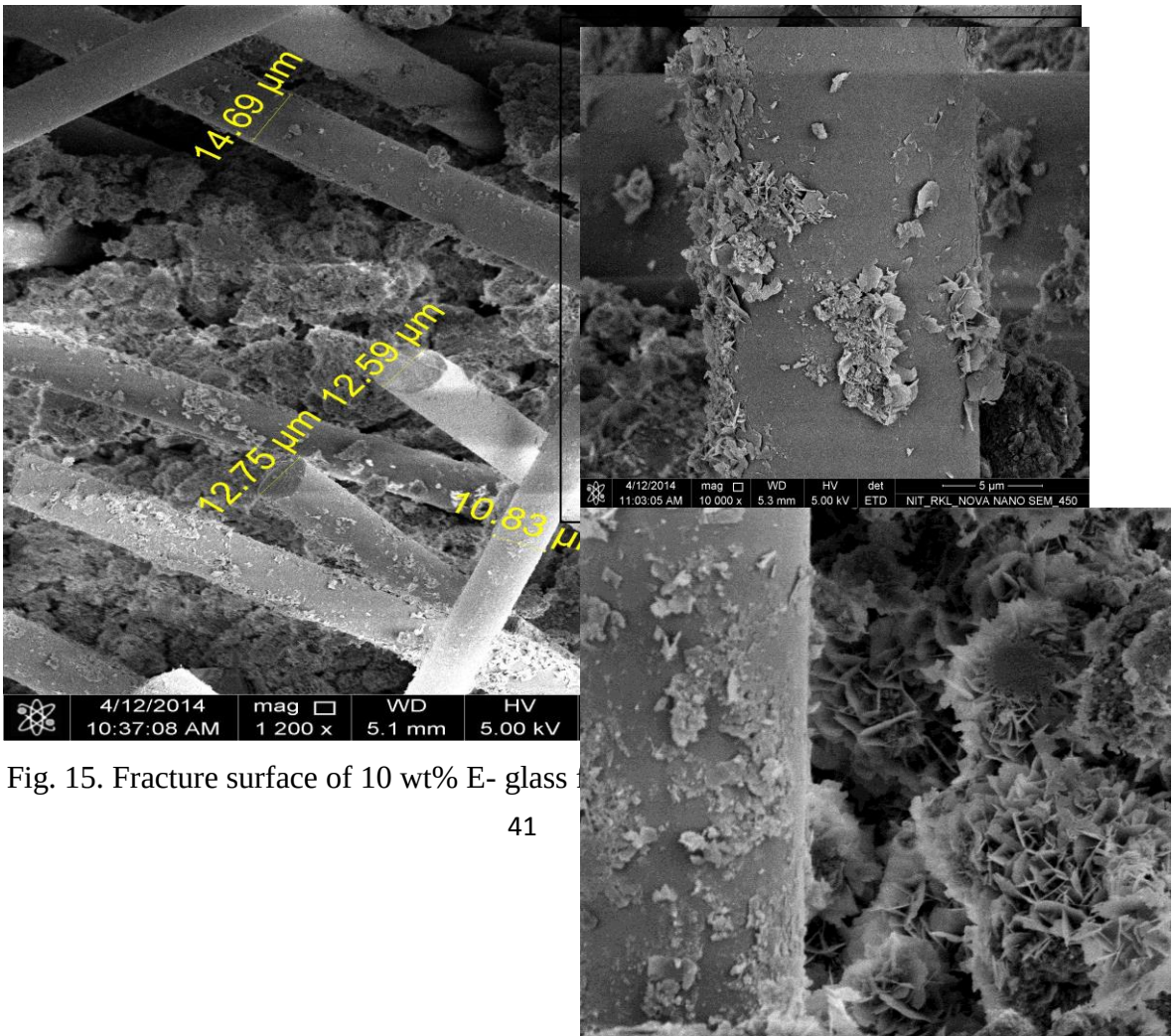

Fig. 15. Fracture surface of 10 wt% E- glass

Figure 15. shows the fracture surface of fibre reinforced Si-doped CPC and as could be observed that addition of 10 weight% of E-glass fibre to Si-doped CPC filled up the voids in the microstructure as well as caused crack deflection to direct inter fiber crack propagation to enhace strength in the composite structure. Also, as evident from the micrograph the growth of apatite crystal on the surface of E-glass fiber could provide stronger interface between glass fiber and CPC crystal and also gave better entanglement between the apatite crystals grown in set CPC grains.

HA crystal grew and provided more entanglement of crystals in the pores of the microstructure to give better compactness with increase in elapsed time in SBF. The lower porosity and greater compactness in the microstructure attributes to greater diametral tensile strength (DTS) values observed in undoped CPC. Another possible explanation of the decrease of strength in Si doped CPC could be the early dissolution of the cement.

Addition of 10 weight% of E-glass fiber to Si-doped α-TCP caused an increase in DTS value because fiber can fill up the voids in the microstructure as well as it can arrest and deflect the crack under diametral tensile stress and increase the strength. Silicon was added to CPC because silicon can increase the bioactivity of the set-cement but at the same time there was decrease in mechanical strength due to silicon addition. This can be compensated by addition of some amount of E-Glass fiber without compromising on its bioactivity.

E-Glass fiber surface is modified with apatite crystal by immersing it into the SBF solution so that the hydroxyapatite crystal generated from the hydrolysis of calcium phosphate cement can easily grow on the top of the apatite layer on the E-Glass fiber and make a strong interface and good compactness in the microstructure.

6. CONCLUSIONS

Effect of Si doping on the properties of α-TCP based calcium phosphate cement was investigated. Doping with Si up to 1 mol% delayed the initial and final setting time of CPC. Si doped CPC exhibited a little slower rate of conversion into HA phase than that of undoped CPC. Diametral tensile strength of both doped and undoped cement increased with increase in elapsed time of CPC in SBF upto 10 days. Undoped CPC showed higher average diametral tensile strength than 1 mol% Si doped CPC at all time points in SBF though incorporation of 1 mol% Si in the cement is supposed to increase its bioactivity. The objective of the present research was to enhance the mechanical strength of Si- doped CPC without compromising on its enhanced bioactivity. Addition of 10 weight percent e-glass fibre onto 1mol% Si doped alpha tricalcium phosphate resulted in almost 1.5 times increase in average diametral tensile strength of CPC.

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